R-, G- and C-banded chromosomes in the Domestic Fowl (Gallus domesticus)

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The Domestic Fowl (Gallus domesticus) is the commonest bird in the world and not surprisingly it is also the most thoroughly investigated in many respects. More than 20 karyotypes have been published and the exact chromosome number (2n = 78) has been determined (POLLOCK and FECHHEIMER 1978). A chromosome linkage map with 40 loci placed on the ten largest chromosomes has also been established (SOMES 1978). Usually, bird chromosomes are divided into a small number of macro- and a large number of microchromosomes. However, the macrochromosomes are rather small, being about equal to the mediumsized chromosomes of the house mouse. Probably, this fact has been an obstacle to more numerous attempts to investigate the interesting evolutionary relationships between birds using chromosome banding techniques. Only a few studies of banded chromosomes of the Domestic Fowl have been published: Q-band (STAHL and VAGNER-CAPODANO 1972), G-band (MATEESCU 1973; and WANG and SHOFFNER 1974) and G- and C-band (STOCK et al. 1974). In this article we present R-, G-and C-band of the Domestic Fowl and discuss theories on the origin of R-band arising from BrdU incorporation.

Material and methods
Skin and muscle tissue from 10-day-old foetuses of chicken were taken for culture in Eagles Medium with HEPES to a final concentration of 2 x 10^{-2} M, supplemented with 20% calf serum. The cells were cultured at 37°C and examined as primary culture. 1 µg/ml colchicine was added 3–4 h before harvesting; after trypsination the cells were treated for 10–15 min in 0.075 M KCl. The cells were fixed in 3:1 methanol-glacial acetic acid with three changes, dropped on wet slides and air dried. For G-bands a modification of the technique of WANG and FEDOROFF (1972) was used (RYTTMAN et al. 1979). Six specimens with good and distinct G-bands were examined.

The technique of SUMNER (1972) was used for C-bands. The treatment time in saturated Ba(OH)₃ solution was 15 min. Three specimens were examined. For R-bands the technique of DUTRILLAUX et al. (1973) was used: 300 µg/ml BrdU was added to the culture medium 7 h before harvesting the cells. The slides were treated in 1/15M phosphate buffer pH 6.70 for at least 1 h, then stained with a solution of acridine orange (3.5 mg/70 ml 1/15M phosphate buffer pH 6.70) rinsed twice in the phosphate buffer and mounted in the same buffer. The metaphases were observed and photographed in a Leitz fluorescent microscope. One specimen was examined.

Results
In Fig.1 the fluorescent colour picture of R-band ed chromosomes obtained after acridine orange
staining is shown. There is a distinct pattern of red and green-yellow bands. Bianchi and Molina (1967) report that the S-phase lasts for 9 hours and that the G2 + prophase last 2.5 hours in bone marrow cells from chicken. A labelling time of 7 hours with BrdU will probably interact with the synthesis at the second half of the S-period even in primary skin fibroblasts. Fig. 2 shows G-banded (left) and R-banded (right) karyotypes. There is a striking correspondence between the positions of the bands with the two methods employed. Black G-bands correspond to red negative R-bands, which appear dark in Fig. 2.

C-banded chromosomes are shown in Fig. 3. The C-banded chicken chromosomes differ from previously described C-banded bird chromosomes (Ryttman et al. 1979) in containing less constitutive heterochromatin in the microchromosomes and centromeres. Note the heavily stained and polymorphic terminal part of the Z-chromosome. This is in agreement with a previously published report (Stock et al. 1974).

In Fig. 4 there is a schematic representation of the G- and R-bands in chromosomes from Domestic Fowl. The pattern of the G-bands is identical with those published by Stock et al. (1974) except that chromosome 3 is subterminal as seen in the quinacrine fluorescent chromosomes in Fig. 2.
Fig. 2A and B. G- and R-banded karyotypes of Domestic Fowl (Gallus domesticus). Only the seven largest microchromosomes are included. G-bands to the left and R-bands to the right. B Quinacrine fluorescent chromosomes. Note the short arm on chromosome 3. — x 2800.
Fig. 3A and B. A C-banded metaphase from Domestic Fowl (*Gallus domesticus*) (male). Note the polymorphic stained terminal part of the Z-chromosome (arrows). B Female sex-chromosomes. — × 2880.

Discussion

The origin of banding patterns of chromosomes after different treatments is not well understood and there are three main theories as to the mechanisms involved: (1) differences in base composition of the DNA, (2) differences in the protein components, and (3) packing differences in the metaphase chromosomes. In addition, the theories can be combined. The chromomere structure consists of a high density of chromatid packing, increased AT content and a high number of repetitive DNA sequences (SCHWARZACHER 1976). The origin of G-bands is ascribed to the chromomere structure of the metaphase chromosome and the ability of thiazin dyes in Giemsa to side-stack on to available DNA (COMINGS 1975). C-banded regions of constitutive heterochromatin have a high resistance to extraction during the C-banding procedure caused by DNA-nuclear matrix protein interaction. The constitutive heterochromatic regions are condensed and enriched with repetitive DNA, and the bases are highly methylated (SCHWARZACHER 1976; COMINGS 1978). But different regions of constitutive heterochromatin, such as centromeres and chromosome ends, can have different proportions of GC and AT and different sequence homology (ARNASON et al. 1978). Chromosomes treated by heat can give R-bands corresponding to low degree of denaturation and regions rich in GC remain double stranded. The same treatment gives a high degree of denaturation and single-strandedness in regions rich in AT. When stained with acridine orange, the single-stranded DNA fluoresces red and double-stranded DNA, green. The method used in our report allows a somewhat different explanation for the red and green stained regions, using the combination of acridine orange and BrdU.

Our hypothesis is that the delayed spiralization caused by the incorporation of BrdU (ZAKHAROW and EGOLINA 1972) gives unorganized (disordered) regions in the nucleic acid chain that differentiate the banding ratio of acridine orange, i.e. one dye molecule per three base pairs for highly organized nucleic acid structures, and one dye molecule per about one base pair for unorganized nucleic acid structures (RIGLER 1969). This is also the explana-
Fig. 4. Schematic representation of the G- and R-bands in chromosomes from Domestic Fowl (*Gallus domesticus*). The G-bands (to the left) are numbered according to the Paris Conference 1971.

Fig. 4, arrows on the R-banded chromosomes indicate negative R-bands difficult or impossible to regard as corresponding to positive G-bands. G-banded chromosomes seem to have more numerous and distinct bands.

Experiments with $^3$H-thymidine incorporation have not revealed any parts of the genome to be late-replicating in birds (Takagi 1972). There is a striking difference between birds and mammals in the chronology of replication when autosomes and sex chromosomes are compared. In contrast to mammals, no late-replicating sex chromosomes in either sex have been found in the chicken (Bianchi and Molina 1967). No difference can be seen on the R-banded pattern of the two Z-chromosomes in Fig. 1. The microchromosomes seem to be replicating simultaneously with the other chromosomes with a few exceptions (Takagi 1972). Comings and Wyandt (1976) have shown that after pre-treatment with heat and staining with acridine orange, the microchromosomes in quail fluoresce green, indicating double-strandedness and GC richness. In Fig. 1, the microchromosomes are either green or red. The red microchromosomes...
indicate a relatively high frequency of AT base-pairs and a late replication pattern. The difference in content of GC and AT base-pairs between the microchromosomes is verified by the different patterns of the C-bands that are shown in Fig. 3. It is well known that heterochromatic parts sometimes are poorly stained by G-banding but are well stained by C-banding. It has been proposed (Comings 1978) that this poor access of the dye depends on an intense condensation of the heterochromatin or masking by proteins. Therefore, the condensation and/or base composition of the microchromosomes seems to be different in different species of birds (Ryttman et al. 1979).

Stock et al. (1974) found a surprising correspondence between the pattern of the G-bands on the three largest chromosomes in three species of the two orders Galliformes and Columbiformes. In this work we can establish a G-band homology for the four largest chromosomes between some close relatives of the chicken in the genus Gallus domesticus and four Larus species (Charadriiformes) except for a short segment on the terminal part of the arm on chromosome 1 which seems to be lacking in the Domestic Fowl, and the more pronounced centromeres on the Larus chromosomes (Ryttman et al. 1979). As already pointed out by Stock et al. (1974), these findings present a problem in interpreting the relationship of karyotyped divergence to phylogeny in birds. Presenting a contrast to this homology, Stock et al. (1974) discuss the difference between some close relatives of the chicken in the morphology of chromosome 2. This lack of homology is confirmed by a study of G-banded chromosomes from three species of the order Galliformes (Ryttman et al., in prep).

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