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# Chromosome homology in birds: banding patterns of the chromosomes of the domestic chicken, ring-necked dove, and domestic pigeon

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*Abstract*

Improved techniques for culturing avian tissue in combination with a trypsin-urea chromosome banding technique has allowed an analysis of chromosome homology in three bird species. Three macrochromosomes of the domestic chicken (Galliformes) showed homology with those of the ring-necked dove and the domestic pigeon (Columbiformes). The ring-necked dove and domestic pigeon displayed similar banding patterns, except that the pigeon possessed an additional four pairs of microchromosomes, which are fused to form two pairs of macrochromosomes in the dove. Both G- and C-banded metaphase chromosomes of the three species are presented, and chromosome evolution and phyletic relations are discussed.

Analyses of avian chromosomes have been difficult because of the high number of microchromosomes. Even now there is uncertainty about the exact diploid numbers for the common avian species. In addition to these problems, avian cell culture has not been as successful as mammalian cell culture.

While many species representing most of the major groups of birds have been karyotyped in recent years, the general similarity of their karyotypes has prevented serious application of karyological data to phylogenetic studies. By utilizing the recent techniques for inducing specific banding patterns on chromosomes and for demonstrating constitutive

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heterochromatin, we believe that the study of avian chromosomes will provide valuable information concerning bird cytogenetics and phyletic relationships. Furthermore, we believe that improved techniques for culturing bird tissues will provide an ample supply of cells for a variety of in vitro studies.

## *Materials and methods*

Biopsy specimens from aorta, lung, and breast muscle of an adult female domestic pigeon *(Columba livia domestica),* a ring-neck dove *(Streptopelia risoria),* and a young adult male domestic chicken *(Gallus domesticus)* were cultured in McCoy's 5a medium supplemented with 30 % fetal calf serum. The minced primaries were incubated at 41° C (chicken, dove) or 39° C (pigeon). Growth at lower temperatures was poor, but in some cases initial colonies were obtained at 37–39° C, and these cultures then grew well at 39–41° C. Air-dried chromosome preparations were made following Colcemid arrest (0.06 μg/ml for 1 h) and a hypotonic solution (3:1 $H_2O$:growth medium) treatment for 10 min. The preparations were made by dropping three to four drops of cell suspension onto slides dipped in distilled water at room temperature, which were then set on end on a blotter to dry. Slides were further dried overnight in a 60–65° C oven.

G-banding patterns were induced by combining the trypsin (SEABRIGHT, 1972) and the urea (SHIRAISHI and YOSIDA, 1972) techniques. The slides were first treated with trypsin for 30 to 60 s, then stained, and finally dipped in urea solution for up to 30 s. The exact time required depends on many factors, and one or two slides should be tried to find the proper timing. The best bands are obtained when the trypsin treatment produces swollen chromosomes with only weak or no bands. Short exposure to urea then produces good bands. The effect of urea is greatly enhanced by pretreatment with trypsin so that only brief treatment times are required. This method combines the swelling typical of trypsin treatment with the fine banding of the urea method, which, in the case of the small chromosomes of birds, is essential. The C-band technique followed the alkaline SSC method of STEFOS and ARRIGHI (1971).

## *Results*

The G-band karyotypes obtained for each species are presented in figs. 1 to 3. The chicken karyotype (fig. 1) contains 6 pairs of macrochromosomes, including the sex pair, and at least 32 pairs of so-called microchromosomes, which range in size from nearly as large as the macrochromosomes to minute. Approximately 12 pairs of the smaller chromosomes can be reliably matched on a comparative basis; and within each karyotype most of the remaining chromosomes can be matched.

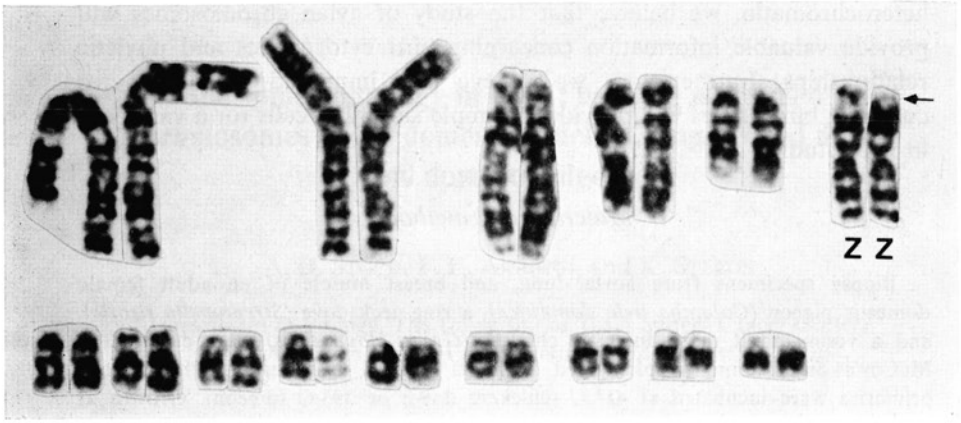


*Fig. 1.* G-banded karyotype of the domestic chicken. Only the first nine pairs of microchromosomes are included.

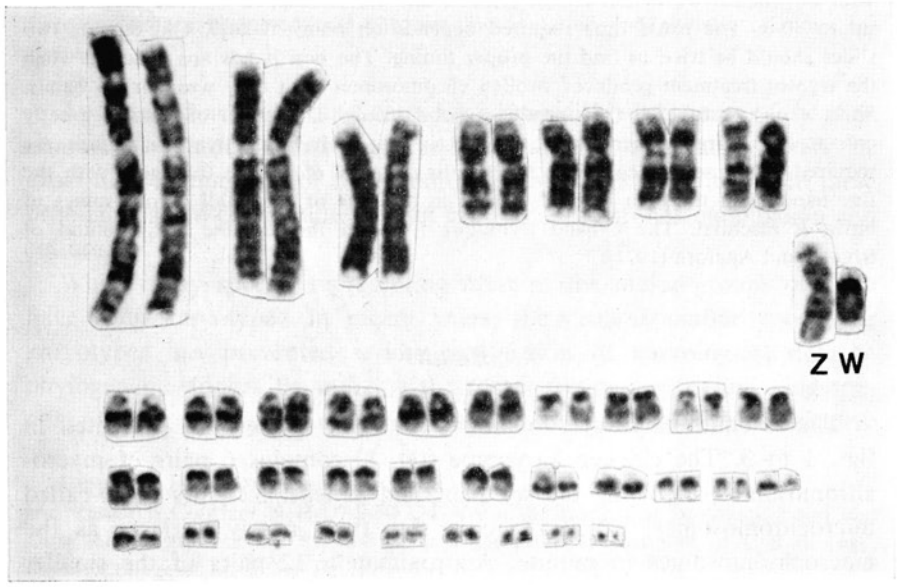


*Fig. 2.* G-banded karyotype of the ring-necked dove (2n=76).

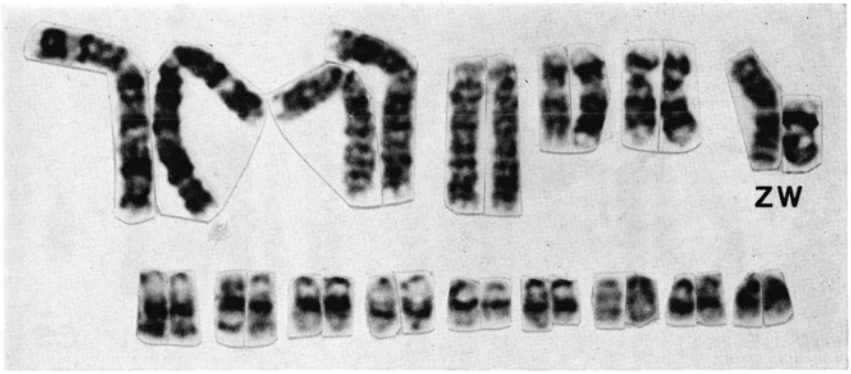


*Fig. 3.* G-banded karyotype of the domestic pigeon. Only the first nine pairs of microchromosomes are included.

The karyotype of the ring-necked dove (fig. 2) contains 8 pairs of macrochromosomes, including the sex pair, and at least 30 pairs of microchromosomes. The size difference between macro- and microchromosomes is distinct, and at least 10 pairs of microchromosomes can be easily recognized for comparative purposes.

The karyotype of the domestic pigeon (fig. 3) contains 6 pairs of macrochromosomes and at least 34 pairs of smaller chromosomes. The size difference between macro- and microchromosomes is not as distinct at that in the ring-necked dove.

Comparison of the G-banding patterns of the chromosomes of these bird species (fig. 4) demonstrates that the three largest pairs of macrochromosomes have similar patterns, whereas two pairs of macrochromosomes of the chicken are unique. Chromosome No. 3 of the chicken is telocentric, whereas that of the other two species is acrocentric. The difference apparently results from a fusing of a microchromosome to the macrochromosome. The G-banding patterns of the pigeon and the dove are similar, except that the pigeon possesses five pairs of macrochromosomes, excluding the sex pair, whereas the dove possesses seven pairs of macrochromosomes, excluding the sex pair. The G-banding patterns indicate that the difference results from the fusion of four pairs of microchromosomes of the pigeon to form two pairs of biarmed macrochromosomes in the dove; thus the smaller four pairs of macrochromosomes of the dove are homologous to two pairs of macrochromosomes and four pairs of microchromosomes of the pigeon.

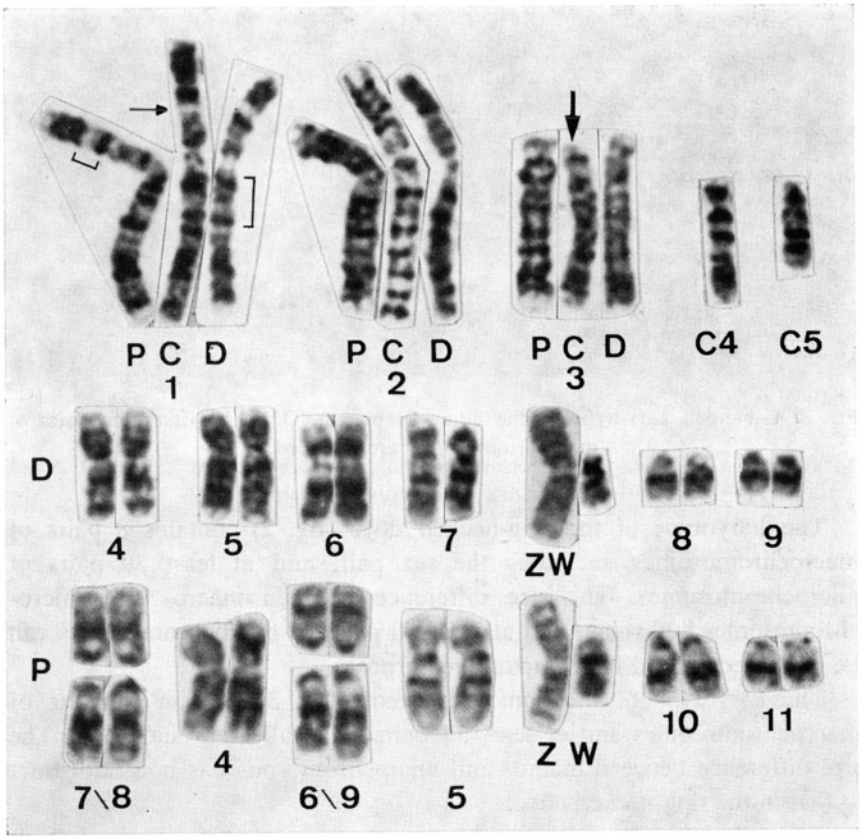


*Fig. 4.* Comparison of the major chromosomes of the chicken (C), dove (D), and pigeon (P). Top row compares the three largest chromosomes of the three species. Chromosomes C4 and C5 are unmatched in the dove and pigeon karyotype. Note the two paracentric inversions in chromosome No. 1 when the chicken chromosome is compared to that of the other two species. The middle and bottom rows of chromosomes compare the macrochromosomes and largest microchromosomes of the dove and pigeon, respectively, and indicate the fusions necessary to transform the pigeon karyotype to that of the dove.

Comparison of the largest chromosome of the three species (fig. 4) demonstrates the presence of two paracentric inversions, one in each arm, between the banding pattern seen in the chicken and that in the dove and pigeon. Whether it is the chicken or the pigeon and dove that possesses the inverted sequence requires study of additional species.

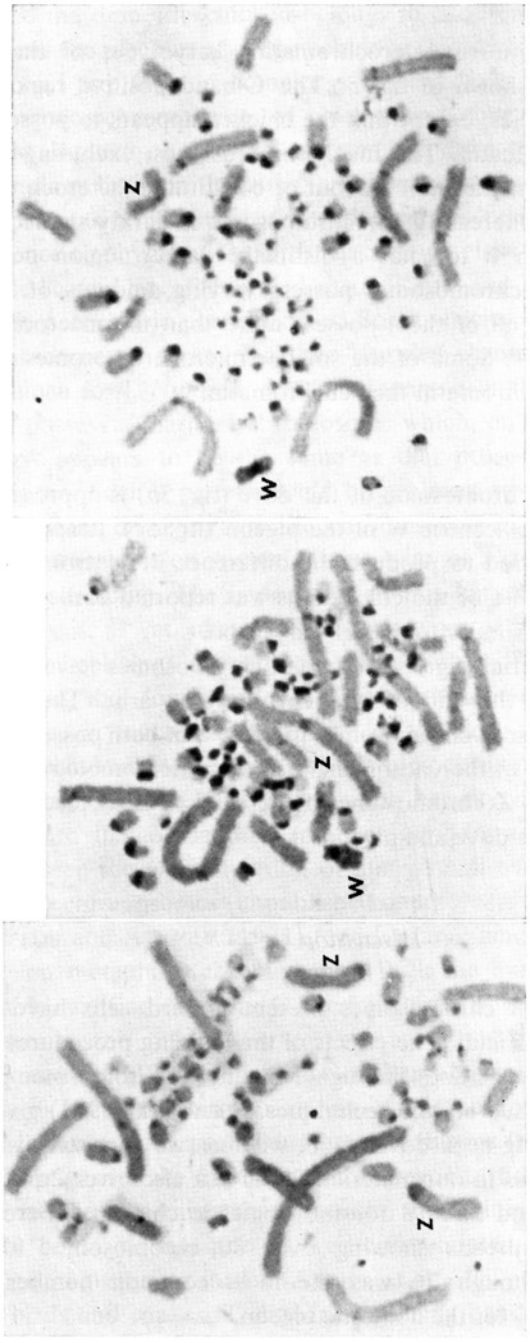


*Fig. 5.* The C-banded metaphase chromosomes of the three bird species. *a.* Chicken metaphase chromosomes showing heteromorphism of the two Z chromosomes. *b.* Dove metaphase chromosomes. *c.* Pigeon metaphase chromosomes.

*C-bands*

The C-band (constitutive heterochromatin) karyotypes of the three species examined are shown in fig. 5. The C-band positive regions are similar in all three species, except that the chicken appears to possess less constitutive heterochromatin. The macrochromosomes, excluding the W chromosome, possess only a small amount of constitutive heterochromatin near the centromere, whereas the W chromosome is darkly stained in all three species, although it too has a distinctly darker region near the centromere. The microchromosomes possess varying amounts of heterochromatin, but almost all of them possess more than the macrochromosomes, excluding the W. Some of the smaller microchromosomes appear to possess more heterochromatin then euchromatin.

*Sex chromosomes*

The acrocentric W chromosome of the dove (fig. 5*b*) is approximately the same size as the metacentric W of the pigeon (fig. 5*c*). It appears that an inversion has occurred to produce the difference in centromere position. The W chromosome of the chicken, as was reported earlier (STEFOS and ARRIGHI, 1971), is similar to that of the pigeon.

In our chicken material (fig. 1) the two Z chromosomes have different G-band patterns near the end of one chromosome arm. The C-band patterns (fig. 5*a*) of these Z chromosomes indicate that both possess constitutive heterochromatin at the end of one arm, but one homolog possesses a greater amount. The Z chromosome of the chicken is metacentric and is similar to the Z of the dove and pigeon.

## *Discussion*

The numerous small chromosomes present in bird cells have made accurate counts very difficult. The effects of the banding procedures make counting more accurate, as does the higher quality of chromosome preparation possible with tissue culture techniques. SHOFFNER (1971) gives the diploid count of the ring-neck dove as 68, whereas we consistently count at least 76 chromosomes in our material. SHOFFNER also gives $2n=80$ for the domestic pigeon and $2n=78$ for the domestic chicken, whereas we have counted good spreads showing over 80 chromosomes for the domestic chicken (although 76 was the most common number) and frequently more than 80 for the domestic pigeon.

Since the domestic chicken belongs to a different order (Galliformes) than does the pigeon and ring-neck dove (Columbiformes), it was surprising to find any G-band homology between them. The differences in the smaller macrochromosomes of chicken and dove and pigeon reflects the phyletic distance of the chicken from the dove and pigeon. The finding of homology between the three largest pairs of macrochromosomes, however, presents a problem in interpreting the relationship of karyotypic divergence to phylogeny in birds, since some close relatives of the chicken lack the second largest biarmed chromosome and have, in place of it, two pairs of acrocentric chromosomes. Some species of pheasants and quail and the domestic turkey lack the biarmed chromosome No. 2, whereas the Guinea fowl, *Numida,* and the Japanese quail, *Coturnix* (SHOFFNER, 1971), possess a biarmed chromosome which, on the basis of gross morphology, appears to be the same as that possessed by chicken, dove, and pigeon. Such a pattern could be achieved by repeated Robertsonian translocations involving specific chromosomes in different lines of birds or by a translocation involving one arm of chromosome No. 2 and a microchromosome. The importance of this karyotypic variation to avian phylogeny is, as yet, unclear. The karyotypes of birds differ by the number and morphology of macrochromosomes and microchromosomes, and our finding that four pairs of "microchromosomes" of the pigeon form two pairs of "macrochromosomes" of the dove invites speculation that macrochromosomes are made up of microchromosomes. In mammals, a parallel can be seen in comparing the karyotype of *Sigmodon hispidus* (2n=52) to that of its close relative *Sigmodon arizonae* (2n=22). Here may be seen the tandem fusion of many small telocentrics to form fewer but larger chromosomes (unpublished data).

STEFOS and ARRIGHI (1971) found a large amount of heterochromatin in pigeon metaphase chromosomes. With the much better material now available, we find that the C-band patterns of the dove and pigeon are similar.

BEÇAK et al. (1964) claimed a close karyological kinship between snakes and birds based on joint possession of microchromosomes, a ZZ♂/ZW♀ sex mechanism, and a relatively greater chiasma frequency. Such an interpretation ignores the fact that lizards, some turtles, and even monotreme mammals possess microchromosomes. In addition, some snakes, particularly boids, do not possess well-differentiated sex chromosomes, and chiasma frequency in turtles (AYRES, et al. 1969, STOCK, 1972) is as high as in birds and snakes. Treatments inducing banding patterns in reptilian

chromosomes are quite different from those that work well for birds (unpublished data). Since there is a wealth of data to support a derivation of snakes from lizards, we feel safe to conclude that any resemblance between snake and bird karyology is convergent in nature.

## *Acknowledgements*

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