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Avian sex chromosomes in the lampbrush form: the ZW lampbrush bivalents from six species of bird

Irina Solovei, Elena Gaginskaya, Nancy Hutchison & Herbert Macgregor

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The ZW bivalent has been identified and characterized in detail in its lampbrush form in oocytes of chicken, quail, turkey, pigeon, chaffinch and sparrow. The sex bivalent in all six species looks like a single highly asymmetrical chromosome. Most of it has the typical lampbrush organization. The terminal one-fifth is relatively thick and condensed and bears only a few pairs of lateral loops: this condensed terminal region is the W chromosome; the part with normal lampbrush morphology is the Z. The two are connected by a single near terminal chiasma. The fine scale morphology and arrangement of loops and markers on Z and W chromosomes are described for each species and lampbrush maps have been constructed. The identification of the lampbrush sex bivalent is based on the following criteria. The asymmetrical chromosome has two centromere regions. In the interstitial region of the asymmetrical chromosome where the junction between Z and W chromosomes is supposed to be, there are telomere-specific loops and telomeric DNA sequences and there is good morphological evidence for the presence of a chiasma. There are W chromosome specific DNA sequences in the region of the asymmetrical lampbrush chromosome that is thought to represent the W. Breed-specific variations in the morphology of the chicken W chromosome with respect to the sizes, numbers and arrangements of axial chromomeres and distributions of specified repeated DNA sequence families have been identified, offering one of the first examples of definitive correlation between a repeat family and a single chromomere. The lampbrush chromosomes of all the birds examined, except quail, terminate in distinctive free hanging loops. These are a novel feature in the sense that at the end of each chromatid there is a large transcription unit terminating in a cluster of telomeric DNA sequences.

Introduction

Lampbrush chromosomes are greatly elongated diplotene bivalents found in the growing oocytes of all animals except mammals, some insects and some reptiles. The large size of these chromosomes and the intense RNA transcription that is taking place simultaneously on hundreds of lampbrush loops makes them particularly useful for locating gene sequences by *in situ* nucleic acid hybridization. This is specially true in animals with small genomes, where many of the mitotic chromosomes are too small to work with. The lampbrush form of even the smallest of chromosomes is easily visible and shows useful linear differentiation. In mitotic metaphases from birds, for example, the largest macrochromosomes rarely exceed 10 μm in length and the smallest microchromosomes are near to the limit of resolution of the light microscope. In the lampbrush form, the lengths of these chromosomes are 20- to 30-fold greater.

The majority of published work concerning the structure and function of lampbrush chromsomes has been centred on the large chromsomes of tailed amphibians (Callan 1986, Macgregor 1987). However, lampbrush chromosomes can also be isolated with relative ease from oocytes of birds and two principal groups of investigators, one in Russia and one in the United States, have studied the lampbrush chromosomes of chicken, quail, turkey, pigeon, sparrow and chaffinch and produced basic cytological descriptions and lampbrush maps, together with preliminary information on locations of certain DNA sequences and some observations on special characteristics and phenomena (Gaginskaya *et al.* 1984, Kropotova & Gaginskaya 1984, Hutchison 1987, Chelysheva *et al.* 1990, Solovei *et al.* 1992).

One of the most striking deficiencies in the literature relating to amphibian lampbrush chromosomes concerns sex chromosomes. The newts that have been particularly favoured for lampbrush studies, *Triturus* and *Notophthalmus*, have poorly differentiated sex chromosomes; in addition, the female is the homogametic sex. Birds, on the other hand, offer excellent opportunities because they not only have cytologically manageable

Irina Solovei and Elena Gaginskaya are at the Biological Institute, University of St Petersburg, St Petersburg, Russia. Nancy Hutchison is at the Fred Hutchinson Cancer Research Center, Seattle, WA, USA. Herbert Macgregor (corresponding author) is at the Department of Zoology, University of Leicester, Leicester LE1 7RH, UK. Tel: (+44) 533 523344, Fax: (+44) 533 523330.

lampbrush chromosomes, albeit much smaller than those of newts, but they also have well differentiated sex chromosomes and the female is the heterogametic sex. Nevertheless, understanding meiotic sex chromosomes in birds has not been easy. Early studies suggested that the W and Z chromosomes of birds remained separate throughout meiosis (Ohno 1961). Kropotova and Gaginskaya (1984) identified an asymmetrical lampbrush macrochromosome which they considered to be a Z univalent, and they specifically noted that they were unable to identify the W chromosome. Hutchison (1987) recognized an asymmetrical Z chromosome, but suggested that the Z and W might be connected with one another by a short terminal pairing region. Studies of zygotene and pachytene chromosomes in chicken demonstrated that the sex chromosomes form an unequal synaptonemal complex with a single recombination nodule located near to the telomeres (Solari 1977, Rahn & Solari 1986, Solari 1992). It therefore seems likely that the asymmetrical macrochromosome described by Kropotova & Gaginskaya and by Hutchison is indeed a sex bivalent, and Solovei *et al.* (1990) have specifically argued that the condensed terminal region that constitutes the supposed short arm of the asymmetrical chromosome is actually the W chromosome.

In this article we present the result of a study of the asymmetrical macrochromosomes from six species of bird. Our observations, together with the results of *in situ* hybridizations with W chromosome-specific repetitive DNA sequences, demonstrate beyond doubt that these chromosomes are ZW bivalents. Both sex chromosomes are described in all six species. Cytological maps are provided for the Z and W chromosomes of chicken (*Gallus gallus domesticus*).

Materials and methods

The birds used for this study were *Gallus gallus domesticus* (commercial Russian line Zarya-17 and English Rhode Island Red crosses), Japanese quail *Coturnix coturnix japonica*, turkey *Meleagris gallopavo*, pigeon *Columba livia*, sparrow *Passer domesticus*, and chaffinch *Fringilla coelebs*. Pigeons and sparrows were caught in the vicinity of St Petersburg. Chaffinches were caught near Kurshskaya Kosa, Kaliningrad, Russia. All birds were sexually mature. Galliformes were on 'point of lay', meaning that they had nearly mature eggs, some complete with their shells, in their oviducts.

The sizes of oocytes used for lampbrush studies differed from species to species: 1–2.5 mm diameter was found to be best for chicken and turkey, 0.5–0.75 mm diameter for Japanese quail, 0.5–1.5 mm diameter for pigeon and 0.25–0.75 mm diameter for sparrow and chaffinch.

Lampbrush chromosomes were isolated manually employing the standard techniques initially developed for the lampbrush chromosomes of amphibians (Gall 1954, Gall *et al.* 1981, Callan *et al.* 1987, Macgregor & Varley 1988), suitably modified for oocytes of birds (Kropotova & Gaginskaya 1984, Hutchison 1987, Solovei *et al.* 1992). Individual oocytes of the required sizes were dissected from the ovary and transferred to '5:1 + phosphate', a medium containing 83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.0 (Gall *et al.* 1981), and the germinal vesicles were removed. The germinal vesicle was immediately transferred to a lampbrush 'observation chamber' (see Macgregor & Varley 1988) containing $\frac{3}{4}$ or $\frac{1}{4}$ strength 5:1 + phosphate with 0.1% formaldehyde. The nuclear envelope was removed manually and the nuclear contents allowed to disperse on the bottom of the observation chamber. The preparations were then centrifuged at 2000–2500 × *g* for 15 min in a Sorvall bench top centrifuge to stick the chromosomes firmly to the slide or coverslip forming the base of the observation chamber (see Macgregor & Varley 1988).

Some preparations were examined directly and unfixed with a phase contrast microscope. Those preparations that were to be used for fluorescent staining (DAPI) and for fluorescence *in situ* hybridization (FISH) were fixed in 2% formaldehyde for 30–60 min and then postfixed in 70% ethanol for several hours or overnight. Preparations that were to be used only for morphological observations were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7, and then postfixed in 70% ethanol. These were stained directly with Coomassie blue R250 (Macgregor & Varley 1988) and then mounted in Canada balsam. Some preparations were stained with DAPI in order to see the detailed arrangement of lampbrush chromomeres, photographed and then subsequently stained with Coomassie blue and made permanent in Canada balsam. Altogether we have examined more than 150 lampbrush sex bivalents from chicken, about 50 from quail, 30 from turkey, 35 from pigeon, seven from sparrows and four from chaffinches.

Lengths of chromosomes and distances between landmark structures on chromosomes were measured on photographs with final print magnifications of about 1500×. The position of each landmark structure was characterized on a scale of 0–100 for each chromosome, according to system suggested by Chelysheva *et al.* (1990).

In situ hybridization

Three plasmids were used as probes: pHuR93, which has in insert of 240 nucleotides that includes the human telomere repeat TTAGGG (Moyzis *et al.* 1988); pUGD1301, which has a 1.2 kb unit of a chicken *Eco*RI repeat sequence family (Saitoh *et al.* 1991) and pAGD0601, which has a 0.7 kb unit of the chicken *Xho*I repeat sequence family (Kodama *et al.* 1987). Probes were labelled by nick translation using biotin-11-dUTP (Boehringer Mannheim). The sizes of the biotinylated probe fragments were shown by electrophoresis to be less than 500 bp. The DNA of the probe was denatured by heating at 75°C for 5 min and was put on slides with chromosomes prewarmed to 37°C. Chromosomal DNA denaturation was performed in 70%

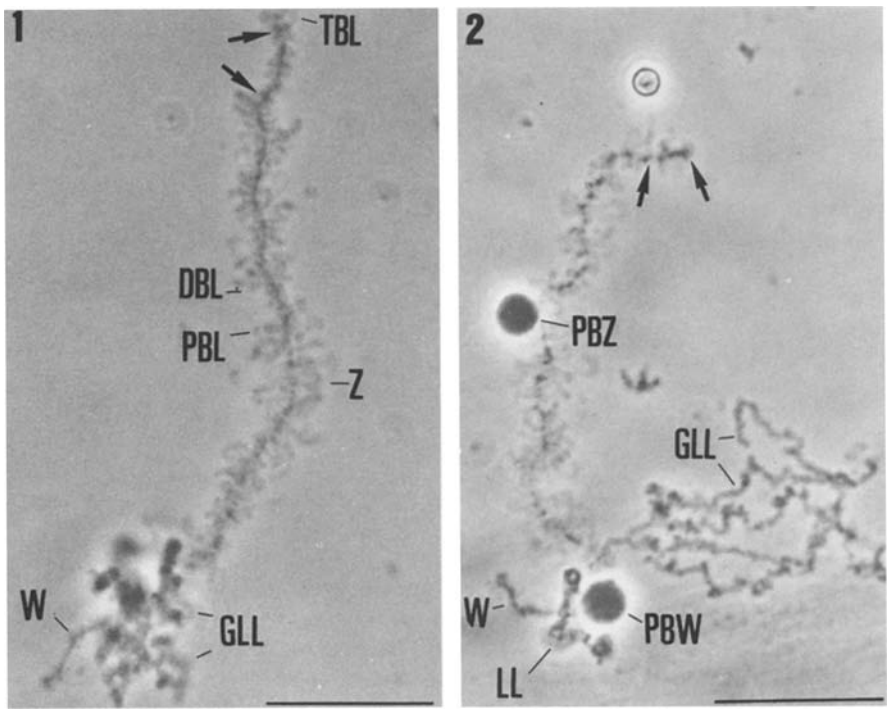
formamide, 2 × SSC, pH 7.0 at 70–75°C. Five microlitres of hybridization mix (50% formamide, 10% dextran sulphate, 2 × SSC, sheared pBR322 DNA) with 40–80 ng labelled probe DNA were placed over each preparation. Preparations were covered with coverslips, sealed with rubber cement and incubated in moist chambers at 37°C overnight. They were then washed in 65% formamide, 7 × SSC at 42°C, followed by washes in 7 × SSC and 4 × SSC at 37°C. For detection of hybrids, slides were incubated with 8 μg/ml FITC conjugated to avidin DN (Vector Laboratories). The chromosomes were stained with DAPI and propidium iodide (PI) and mounted in DABCO antifade media. Some control slides were not denatured before incubation in the hybridization mix. Others were treated with ribonuclease A before hybridization. In order to check that the hybridization procedure was working properly, we placed a number of chicken erythrocytes on each slide alongside the lampbrush preparation(s). Hybrids were visualized with a fluorescence microscope and imaged using the Biorad MRC600 Confocal Scanning Laser Attachment to a Zeiss Axiovert microscope. The grey-scale images were false coloured using the 'autumn look-up table' prior to being hard copied via a Sony UP-3000P colour video printer.

Observations

In all six species studied, the lampbrush chromosomes are of three distinct morphological types. The larger bivalents are basically similar in structure and appearance to lampbrush bivalents that have been described elsewhere in amphibians and reptiles (Callan 1986). They are symmetrical and the half bivalents, joined by chiasmata, show clear homology with respect to distinctive loops and other landmark structures. The microbivalents appear as miniature lampbrush bivalents with half-bivalents associated by just a single chiasma. Their appearance depends on the position of the chiasma: if it is interstitial then the bivalent is cross-shaped, if it is sub-telomeric the bivalent is V-shaped and if the chiasma is terminal the bivalent is rod-shaped. However microbivalents are always clearly symmetrical.

The third kind of lampbrush chromosome is one that consistently looks like a single highly asymmetrical univalent (Figures 1 and 2). The greater part of this structure has a typical lampbrush organization but the terminal one-fifth is relatively thick and condensed and bears only a few pairs of small lateral loops. The border between these two kinds of axis organization is marked by a few large conspicuous loops (GLL on Figures 1 and 2). In the pigeon, sparrow and chaffinch this asymmetrical chromosome always carries two attached protein bodies, similar to those described by Gaginskaya (1972), Tsvetkov & Gaginskaya (1983) & Khutinaeva *et al.* (1989) (PB on Figure 2).

At an early stage in this study we formed the opinion that the asymmetrical chromosome was the sex bivalent, that the region with typical lampbrush organization represents the Z chromosome (Z on Figures 1 & 2), that the condensed terminal region (labelled W in Figures 1 & 2) was the W chromosome and that the Z and W



Figures 1 & 2. Phase-contrast micrographs of sex bivalents from **1** chicken and **2** pigeon. Z and W, axes of Z- and W-LBCs; GLL, giant lumpy loops in the region of chiasma between Z and W axes; in the chicken (**1**) GLLs mask the chiasma, in pigeon (**2**) they are unfolded and it is possible to see all four of them; PB, protein bodies on Z-LBC (PBZ) and on W-LBC (PBW); LL, lumpy loops on the pigeon W-LBC near the PB-locus; TBL, telomere bow-like loops; DBL and PBL mark the centromere region on chicken Z; arrows show the condensed region on the free arm of Z-chromosomes. Scale bars = 20 μm.

chromosomes were connected by a terminal or near terminal chiasma. This view was based on three principal observations which were made on lampbrush chromosomes from pigeon, sparrow and chaffinch. Our conclusions from these observations later proved to be equally applicable to chicken, turkey and quail.

First, protein bodies are associated with the centromere regions of all the lampbrush chromosomes in pigeon, sparrow and chaffinch. The fact that the putative sex bivalent carries two of these protein bodies suggests that it includes two centromere regions. Second, lampbrush chromosomes from all species that are included in this study, except quail, have a common and conspicuous feature: each chromosome bears one pair of telomeric loops with distinctive appearances. For example, LBC from pigeon, sparrow or chaffinch all have telomeric giant lumpy loops (GLL). Loops so long and so enriched with RNP-matrix as GLLs have never been seen in the other parts of bird LBCs (Khutinaeva *et al.* 1989, Chelysheva *et al.* 1990). Chicken LBCs also have telomeric bow-like loops (TBL) and small telomeric lumpy loops in addition to GLLs. We considered that the presence of two pairs of GLLs in the interstitial part of asymmetrical pseudounivalent (Figure 3) indicated the existence of the two ends of chromosomes in this part, associated through a single terminal or near terminal chiasma. Third, specifically in pigeon, the proximal end of the condensed region that we supposed to be the W chromosome shows an unmistakable chiasmate association with the remainder of the chromosome which we supposed to be the Z. This chiasmate association is much less obvious in the other species of bird that we have examined.

The sex bivalent of chicken (*Gallus gallus domesticus*)

The fully extended Z lampbrush chromosome in this species is 70–100 μm long in fixed and stained preparations. There are three distinct regions along the length of the chromosome (Figures 4–7; Figure 19A, D). Region I occupies about 17% of chromosome and lies at the free end of it. It is characterized by closely packed chromomeres bearing uniformly short loops that extend only 2–3 μm from the chromosome axis. Region II occupies the central part of the chromosome and constitutes about 60% of it. It is characterized by large chromomeres that are more widely spaced along the axis and loops that are at least twice as long as those in region I. In region III, which is about 25% of chromosome, the axis consists of very small chromomeres bearing loops of variable length. Among these small chromomeres, in the distal part of region III, there is one large chromomere, that stains brightly with DAPI (Figures 4, 6, 8–14).

The free telomere of the Z carries distinctive bow-like loops (TBLs) that differ from other lateral loops on account of their 'fluffy' appearance (Figures 1, 3, 5, 7, 19A). The long arm telomeres of all the macro-autosomes in chicken have similar TBLs (Chelysheva *et al.* 1990). The loops that mark the outer limits of region II (marker loops, ML1 and ML2) are useful landmark structures only because they are relatively long in comparison with the series of loops nearby. Other marker loops (MLs 3–5), characterized by being distinctly longer than the majority of loops on the chromosome, are situated in regions II & III (Figures 3, 5, 7, 19A).

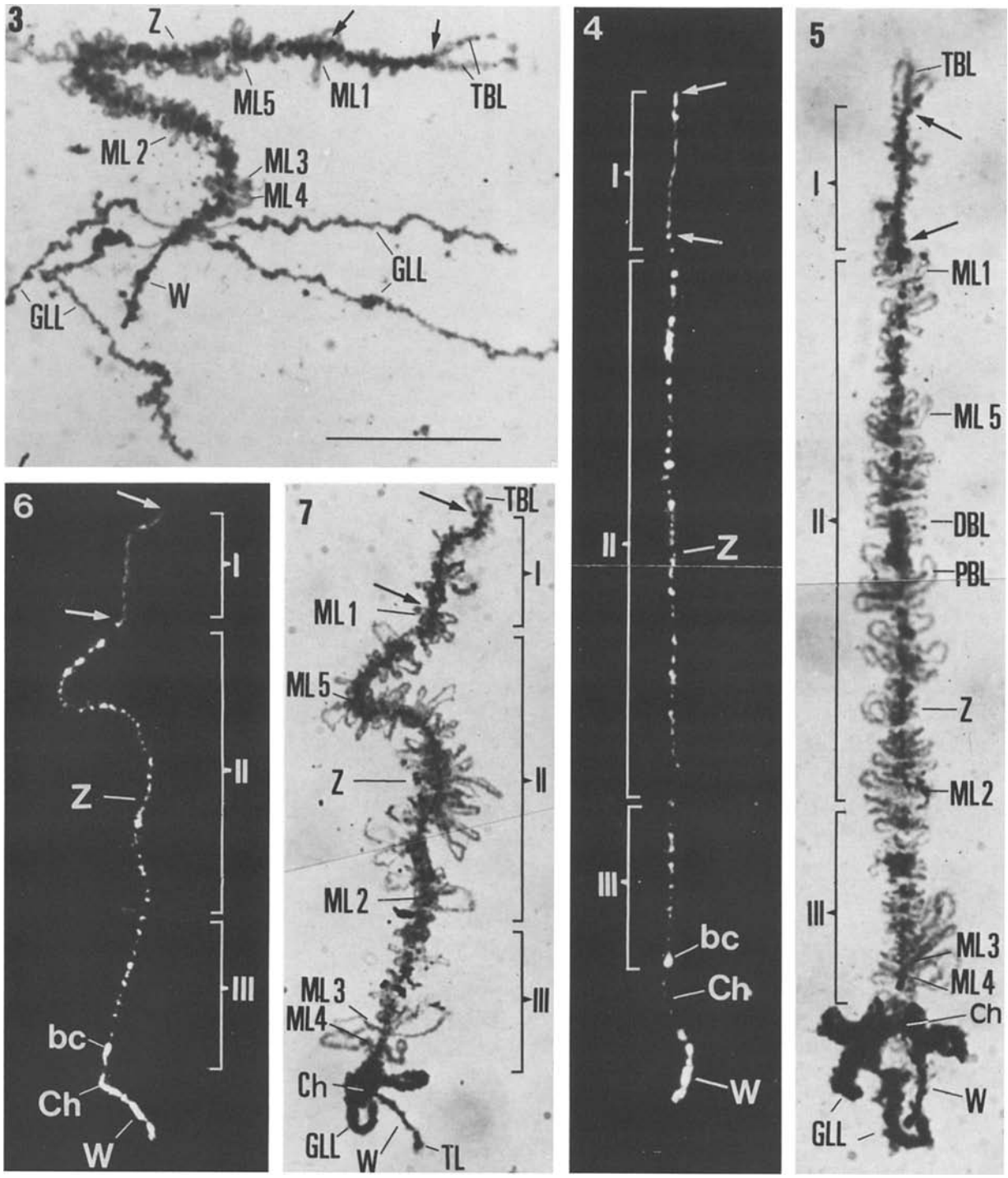
We have previously demonstrated that the centromere on the chicken LBC lies within a small region, between markers PBL and DBL (proximal and distal boundary loops, correspondingly), that is characterized by loops which are relatively small and compact in appearance (Chelysheva *et al.* 1990). In about 50% of all Z-LBCs this region (PBL-DBL on Figure 1; Figures 5 & 19A) is clearly distinguishable and if the centromere is indeed located here, then the centromere index (CI) of the Z-LBC would be 0.45, with the arm that is associated with the W chromosome being the longer of the two.

Although not all the markers mentioned above are seen in every chicken, nor even in every Z chromosome from one chicken, they do nonetheless form a good basis for the construction of a cytological map of the chicken Z-LBC (Figure 19D).

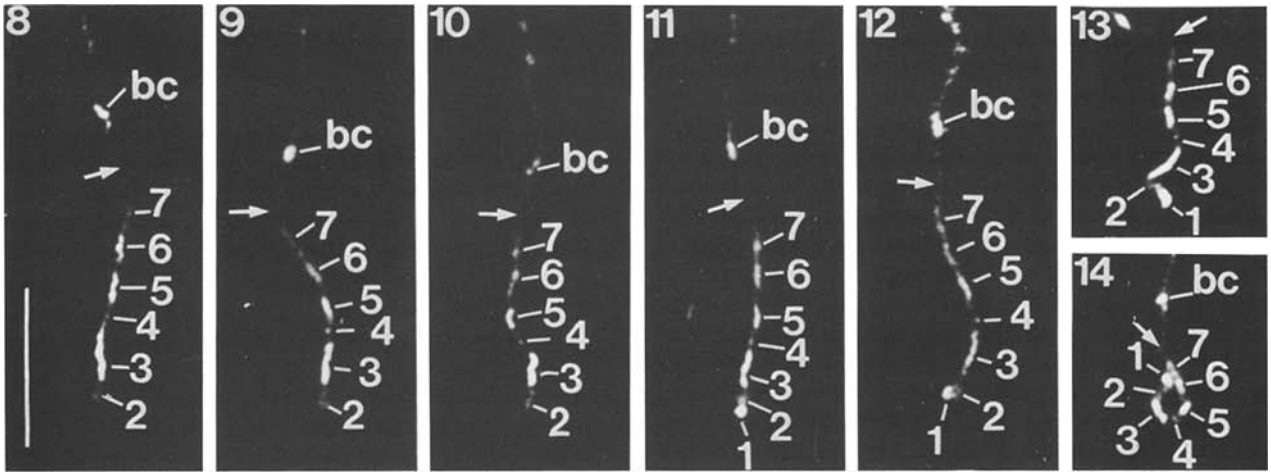
The W-LBC is only 9–14 μm long. It is formed by a dense axis with minute loops that are hard to resolve with the light microscope. The free telomere bears a pair of small granules that are probably very small LLs (Figures 7 & 19A-C). The axis of W-LBC consists of several densely packed chromomeres that are larger and brighter, after staining with DAPI, than the chromomeres in Z-LBC (Figures 4 & 6). In the condensed sex bivalents these chromomeres fuse and form a bar-like structure. At the stage of fully developed LBCs, from the oocytes 0.5–1.5 mm diameter, the arrangement of chromomeres in the W-LBC is absolutely consistent from one preparation to another and each chromomere is individually recognizable.

There are two kinds of W-LBC in chicken: those with six visible chromomeres (Figures 8–10) and those with seven (Figures 11–14). The difference between them resides in the presence or absence of a very bright chromomere at the extreme end of free W arm. The other six chromomeres have the same appearance and arrangement in both kinds of W-LBCs (Figures 8–14, 19E, F). Occasionally the chromomeres on the W chromosome have an elongated and twisted appearance (Figures 8, 12, 19G), as if a higher order of coiling had been partially relaxed, making the whole chromosome much longer than usual.

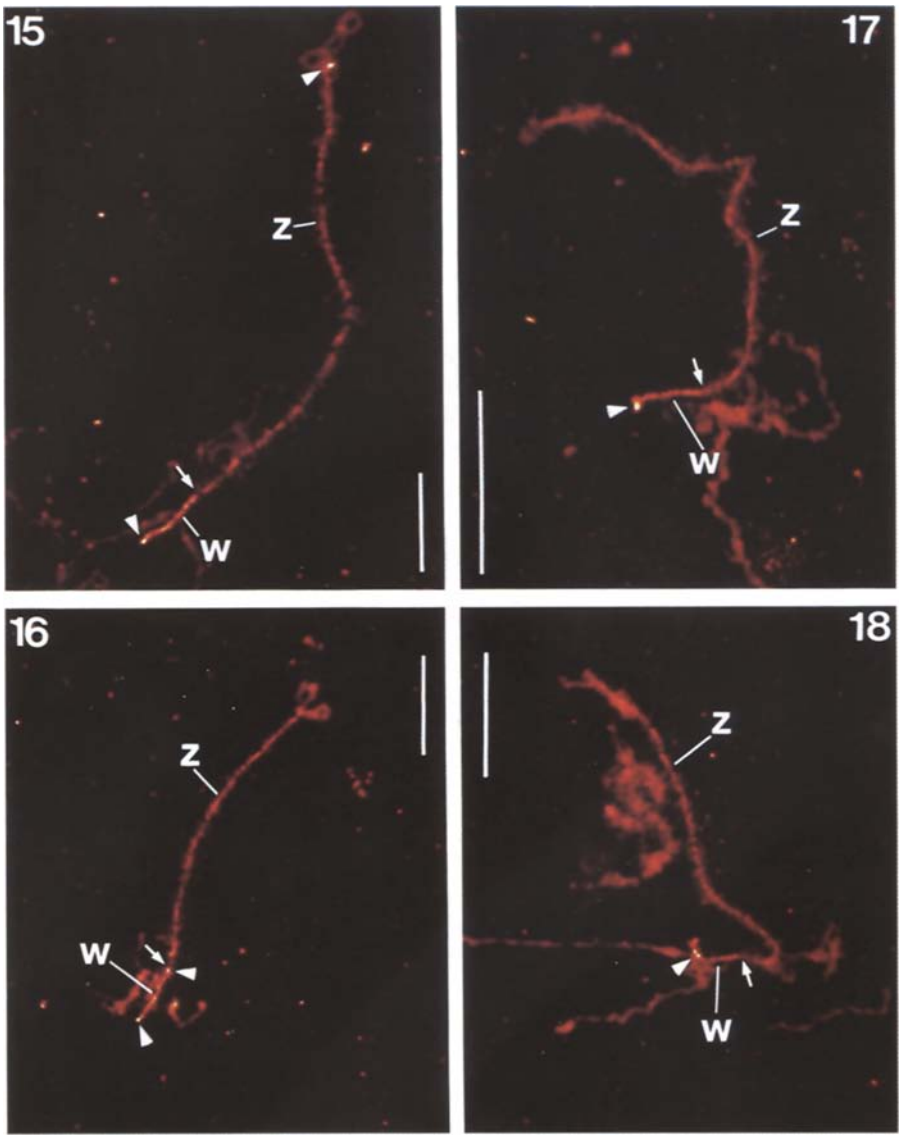
The position of the chiasma is marked by two pairs of giant lumpy loops (GLL) stretching out from the telomeres of the Z and W chromosomes. GLLs often shrink into globules which may mask the W-LBC and the chiasma (Figures 1 & 19C). When these loops are more or less condensed (Figures 5, 7, 19B), one can discern a gradient of RNP matrix from one end of the loop to the other. When they are completely unfolded (Figures 3 & 19A), they may be as long as 70–90 μm and no gradient is seen. The ends of all or only some of GLLs very often hang free, rather than being tacked back onto the chromosome to form typical closed loops (Figures 3 & 19A). In such cases,



Figures 3–7. Sex bivalents from chicken after staining with Coomassie R250 (3) or DAPI (4 and 6) and then restaining with Coomassie (5 and 7). I, II, III, regions of Z-LBC; marker loops on Z-chromosome (TBL, ML1–5, GLLs) are indicated on 3, 5 and 7; Z and W, axis of Z-LBC and W-LBC; the condensed region I in figures 4 and 6 is marked by arrows; bc, the large bright chromomere in region III; TL, telomere lumpy loops on free end of W-LBC; ch, the estimated position of the chiasma between the Z and W axes. Scale bar on 3 is 20 μm and applies to all figures in this set.



Figures 8–14. Appearance of the chicken W-LBC axis after staining with DAPI. W-chromosomes with six chromomeres from three different chickens are shown in Figures 8, 9 & 10; W-chromosomes with 7 chromomeres, each from a different chicken, are shown in figures 11, 12, 13 and 14. All chromomeres on W are numbered; bc, bright chromomere in region III of the Z-chromosome; arrows show the estimated positions of the chiasmata. Scale bar in Figure 8 = 10 μm and applies to all seven figures in this set.



Figures 15–18. Results of FISH with telomere probe pHuR93 (15 & 16) and with W-specific repeats and the lampbrush sex bivalent of chicken: *Eco*RI family, pUGD1301 (17) and *Xho*I family, pAGD0601 (18). Z and W, axes of Z- and W-LBCs; arrows indicate chiasmata; arrowheads show sites of labelling with FITC-labelled avidin. Scale bars are 25 μm.

the true telomeres of each of the chromatids that form the LBC must lie at the extreme tips of the free-hanging loops. As a rule, the Z/W chiasma is in the axis of the chromosome, very close to the telomere and near to the chromomere from which the GLLs originate. It is impossible to see the cross-over point between the axes of the two chromosomes. Usually, the axes of the Z and W chromosomes lie at an angle to one another, which we think is indirect evidence of the presence of a chiasma (Figures 6–9, 13, 14). On at least two occasions, however, the chiasma was situated about 2 μm from the last chromomere on the chromosome axis and we were able to observe it.

Fluorescence *in situ* hybridization (FISH) with the chicken ZW bivalent

In view of the very small size of structures in the region of the border between the two sex chromosomes and the masking of this region by GLLs, we have tried to demonstrate the existence of telomeres in this region by FISH with a human telomere probe. After DNA/DNA hybridization the free telomeres of Z and W-LBCs, together with those of all autosomal bivalents, were clearly labelled with small fluorescent spots (Figure 15). Six sets of LBCs have been used for hybridization with this probe. In three of them, ZW bivalents were labelled near the putative point of ZW association, in the region where GLLs are situated (Figure 16).

In search of additional evidence of the limits of the W-chromosome in the ZW-bivalent, we have employed FISH with W-specific repeated DNA sequences. All the sex bivalents which were used for these experiments had W-LBCs with seven chromomeres.

The probe pUGD1301, which included a 1.2 kb unit of *Eco*RI repeat family, labelled the W chromosome at the same locus in all the seven sex bivalents used in our study. The label was restricted to chromomere 1, the terminal one on the free arm of W-chromosome (Figure 17). Four sets of LBCs were used for FISH with the probe pAGD0601 which included the 0.7 kb unit of the *Xho*I repeat family. All four W-LBC demonstrated an oblong spot or two very close, but clearly separate, spots of label on the largest elongated chromomere number 3 (Figure 18). Hybridization with all probes used in this study, pHuR93, pUGD1301 and pAGD0601, was carried out in three ways: DNA/RNA transcript, DNA/DNA + RNA transcript, and DNA/DNA. Specific labelling was observed only in the case of DNA/DNA + RNA transcript and DNA/DNA hybridization. The absence of label in DNA/RNA transcript FISH preparations suggests that the repeated DNA sequences represented by the probes may not be transcribed during the LBC-stage.

The sex bivalent of the turkey (*Meleagris gallopavo*)

The ZW bivalent of the turkey is basically similar to that of the chicken. The typically lampbrush axis of the Z

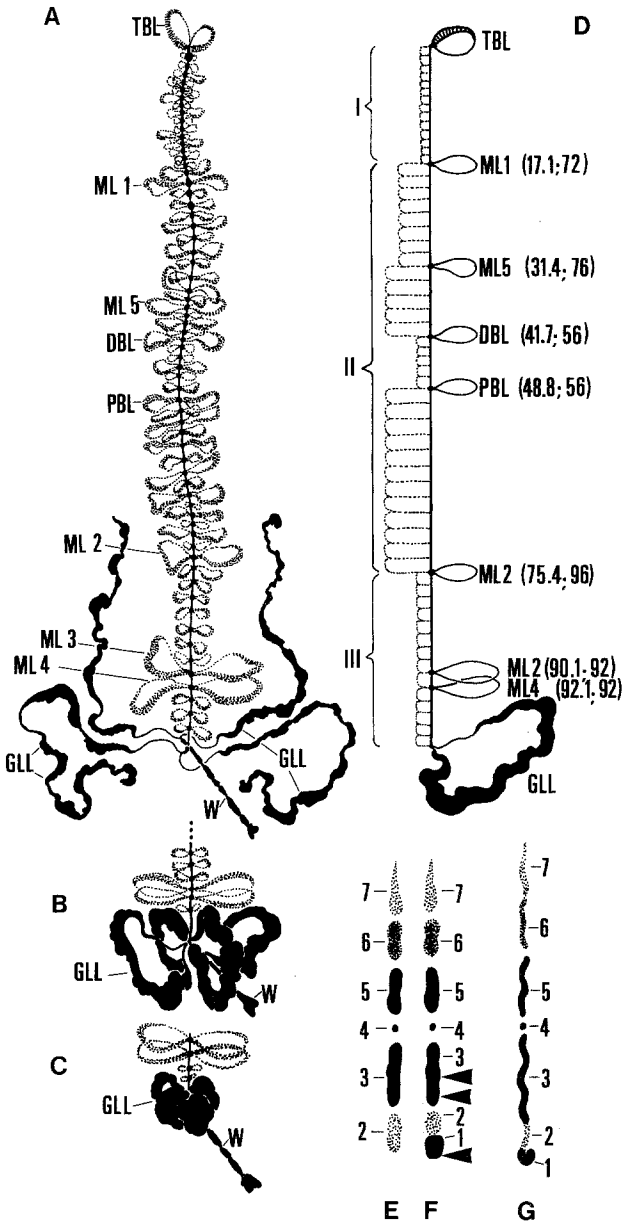
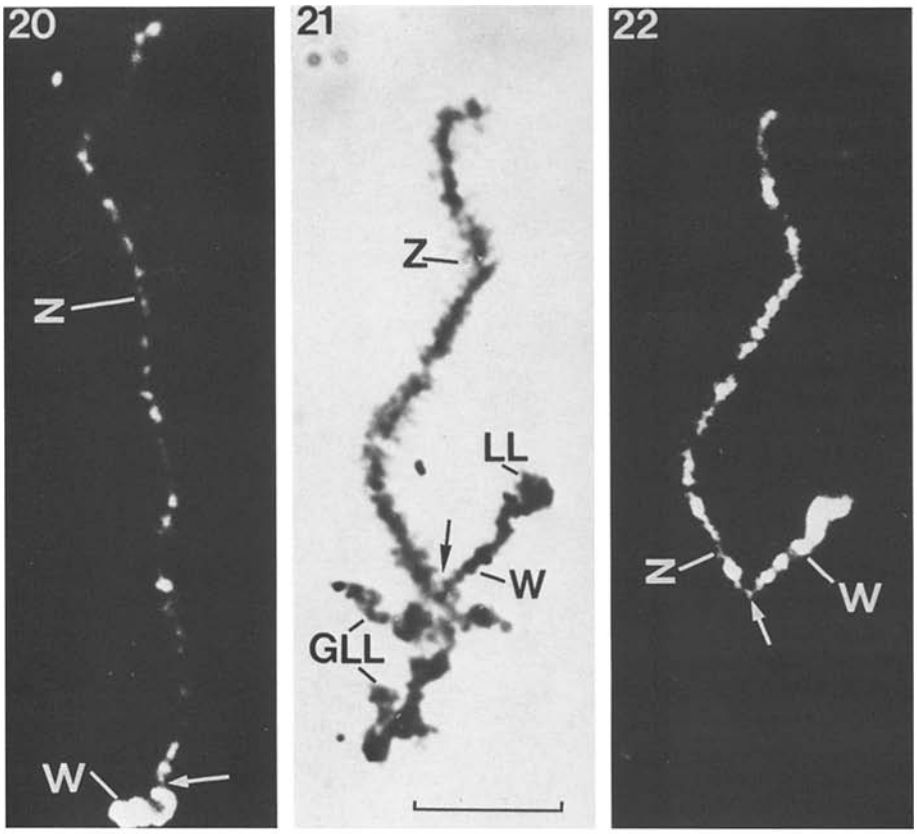
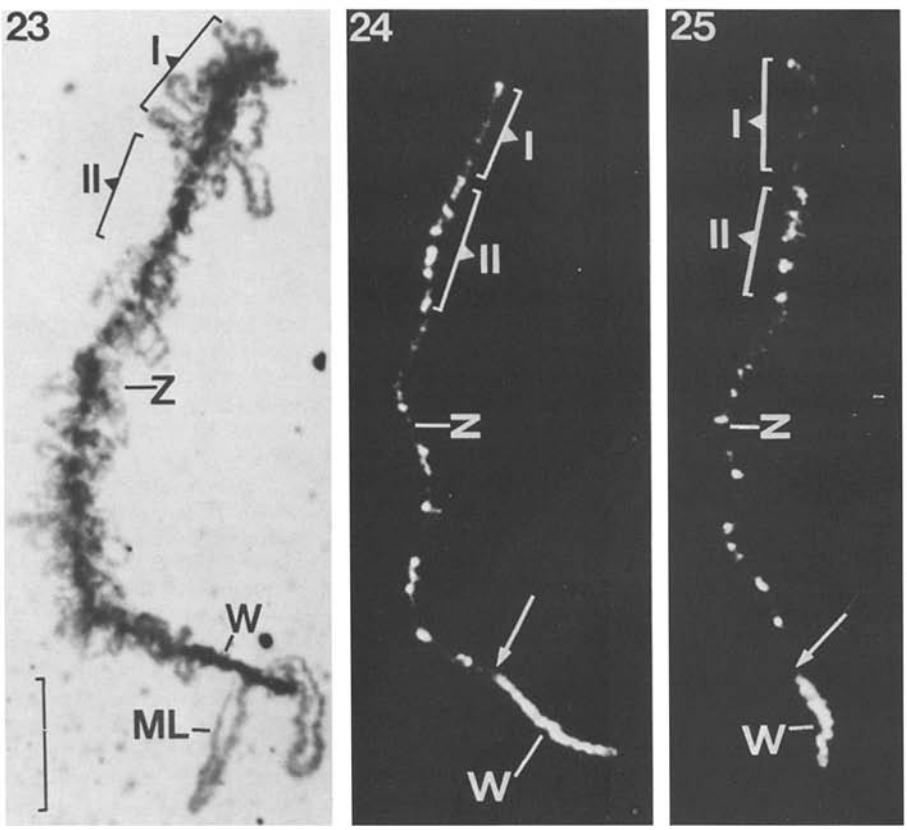


Figure 19. Organization of the chicken sex bivalent. Schematic drawing of whole ZW bivalent (**A**) and appearance of its chiasmate region with GLLs in different states of condensation (**B** and **C**). Marker loops on Z-chromosome, TBL, ML1–5, PBL, DBL, GLLs, and axis of W-LBC, W, are indicated. **D,** working map of Z-LBC. I, II, III, regions of Z-LBC. Co-ordinates of each marker loop and the percentage frequency of occurrence of chromosomes in which each marker could be clearly identified ($n = 25$) are given in brackets. **E, F, G,** diagram of the positions and relative brightness of the chromomeres in W-LBC having six (E) and seven (F, G) chromomeres; in G, the axis of W chromosome is partially relaxed. All chromomeres are numbered; differences in chromomere brightness are demonstrated by black colour for bright ones and by strong or weak shading for dimmer ones; arrowhead indicates the localization of label after FISH with pUGD1301 probe (*Eco*RI family); double arrowhead shows site of hybridization pAGD0601 probe (*Xho*I family).



Figures 20–22. Sex bivalents from turkey. 20, appearance of Z- and W-axis after staining with DAPI. 21, ZW bivalent after staining with DAPI and 22, the same bivalent after restaining with Coomassie. Z and W indicate axis of Z- and W-LBC; GLLs, giant lumpy loops in the region of chiasma; LL, telomere lumpy loops on the free end of W; chiasmata are indicated by arrows. Scale bar on 21 is 10 μm, also applies to 20 and 22.



Figures 23–25. Sex bivalents from quail after staining with Coomassie (23) and after staining with DAPI (24 and 25). Z, W, axis of Z- and W-chromosomes; I, II, two most pronounced regions of Z-chromosome; ML, long marker loops on the W-chromosome; putative chiasma of bivalents is indicated by arrow.

connects with the condensed and loopless axis of the W by a nearly terminal chiasma. Both chromosomes bear one pair of terminal GLLs that originate very near to the chiasma (Figures 21, 22, 26 C).

In many preparations, the W chromosome, together with its GLLs, is compacted into a single large dense globule that stains very brightly with DAPI (Figure 20) and only rarely can its axis be seen (Figures 21 & 26D). We have never seen a completely unfolded W chromosome, but from the partially unfolded ones we can be certain that the entire chromosome is much longer than its counterpart in chicken and that it has lumpy loops (LL) at its free end (Figures 21 & 26C, D). Studies of mitotic chromosomes have also shown that the turkey W chromosome is considerably longer than that of the chicken (Mayr & Auer 1988; Mayr *et al.* 1990).

The sex bivalent of the quail (*Coturnix coturnix japonica*)

Unlike all the other species that are included in this study there are no prominent telomere loops on LBCs in quail. Therefore, although the sex bivalent can easily be recognized, the exact position of the chiasma can be neither seen nor estimated on morphological grounds. Examination of DAPI stained preparations, however, together with knowledge of general patterns seen on the ZW bivalents of other birds, suggests that the region of chromomeric axis bearing loops of variable lengths belongs to the Z chromosome, whereas the W-chromosome comprises the small portion of the bivalent that has a strongly condensed and almost loopless axis (Figures 23–25; Figure 26A). In permanent, fixed and stained preparations, the Z chromosome is 60–70 μm long. The W is 8–15 μm long and carries a single pair of long loops about one-third of the way along from its free end. The free end terminates in a single pair of very small loops (Figures 23 & 26A).

There is a consistent arrangement of lampbrush loops on the Z chromosomes comprising three regions carrying long loops and three with uniformly short ones. The most conspicuous of these are the sub-telomeric region at the free end of the Z, characterized by small chromomeres and very long loops, and the neighbouring proximal region, consisting of 8–10 large, closely packed chromomeres with short loops (Figures 23–25; Figure 26A). In comparison with other species, the axis of the W-LBC looks remarkably wide, although this feature probably results from coiling of a thinner and longer axis. Evidence of coiling is sometimes seen in partially unwound chromosomes stained with DAPI (Figures 24–26A, B).

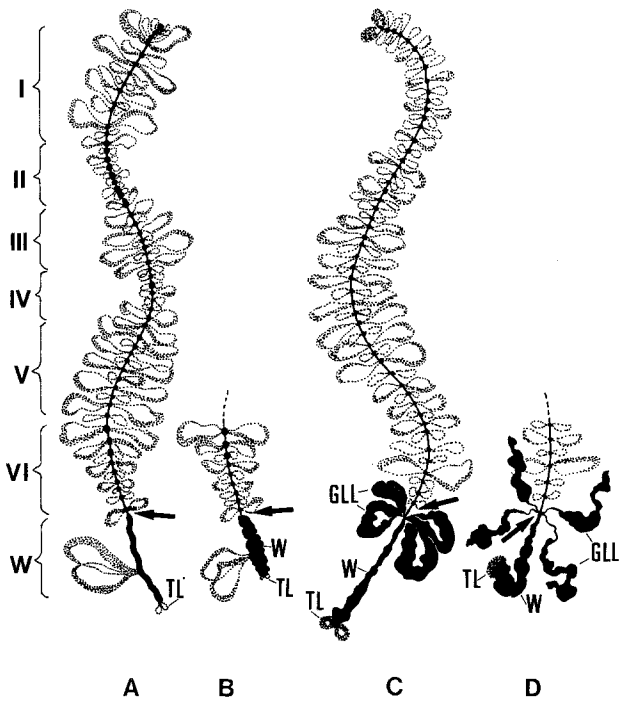


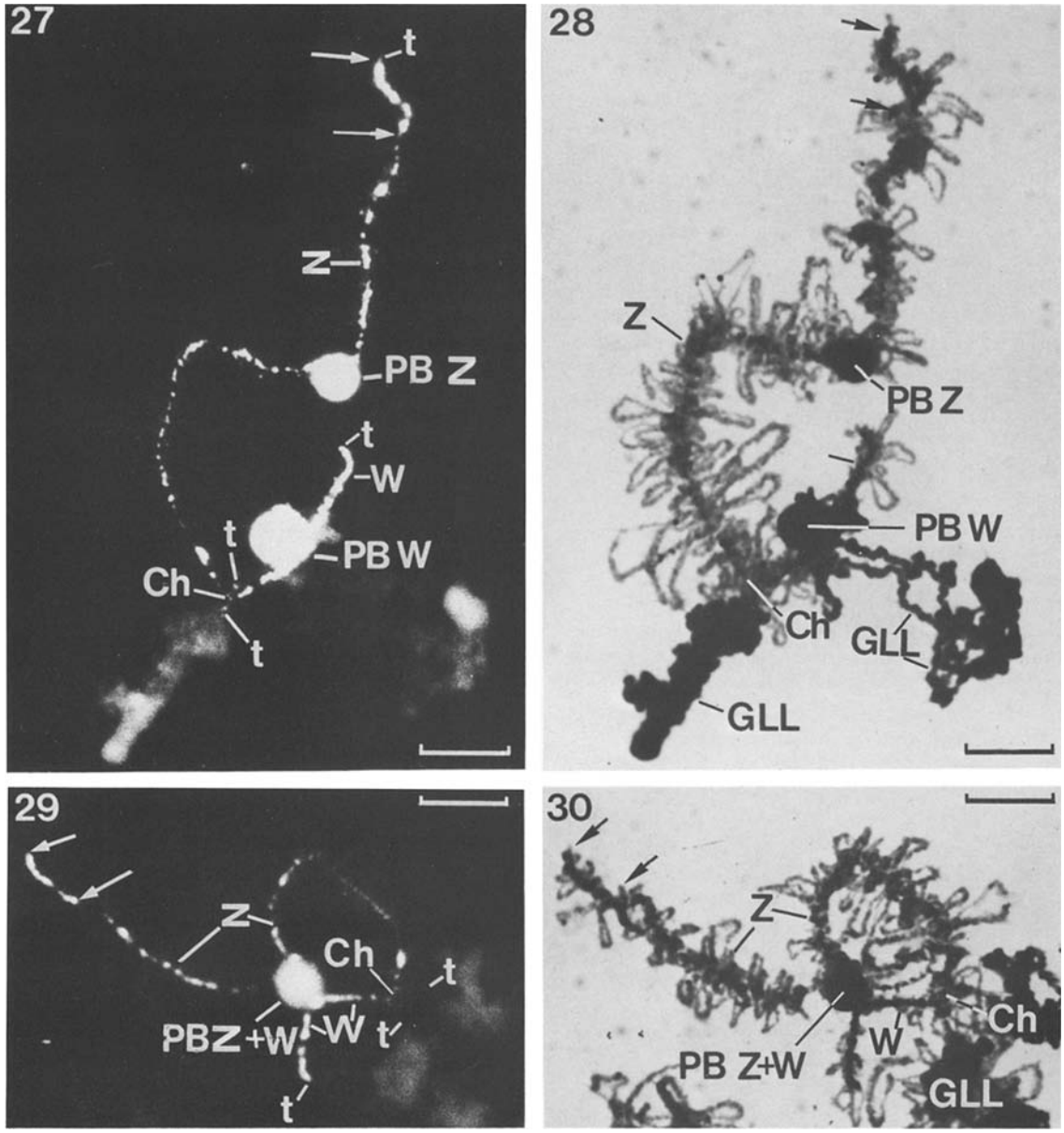
Figure 26. Schematic drawing of ZW bivalents from quail (**A** and **B**) and turkey (**C** and **D**). The W-axis is partially relaxed on a and c; on b and d the W chromosomes are represented in the more usual, condensed state. I–VI, regions with different lengths of loops in quail Z-LBC; Z, W, axis of Z- and W-chromosomes: GLL, giant lumpy loops near the chiasma locus; TL, telomeric loops on the free arm of W-chromosome.

The sex bivalent of the domestic pigeon (*Columba livia*)

The Z chromosome of pigeon does not differ markedly from the autosomal LBCs. Its axis shows up in DAPI-stained preparations as a row of distinct chromomeres of varying sizes and these carry normal looking lampbrush loops of diverse lengths. Towards the free end of the Z, there is a short but consistently distinguishable region of uniformly short loops associated with large chromomeres that stain brightly with DAPI (Figures 2, 27, 28, 33). The axis of the W chromosome consists of large chromomeres that stain brightly with DAPI and are sometimes fused together to form a bar-like structure (Figure 27). The W chromosome has relatively few loops and those that are present are quite small (Figures 28, 30, 33). In the vicinity of the protein body (PB), the W chromosome carries a pair of conspicuous lumpy loops (LLs) that are sometimes fused into a single lump of material (Figures 2 & 33).

One perfectly round protein body (PB) is located approximately in the middle of each of the Z and W chromosomes (Figures 2, 27, 28, 33). PBs range in diameter from 1 to 10 μm. The PBs of homologous and non-homologous half-bivalents often fuse with one another. When those of the Z and W chromosomes fuse (Figures 29 & 30) and, more so, when they fuse with the PBs of other chromosomes, identification of the sex bivalent is considerably more difficult.

Unlike the other LBCs of the pigeon set, the Z and W chromosomes have GLLs only at the ends that take part in chiasma formation. There are no GLLs at the free ends of the chromosomes. As a rule, the chiasma of the sex bivalent is distinctly sub-telomeric and there is a short stretch of chromosome axis between the chiasma and the chromomere from which the GLLs originate (Figures 27,



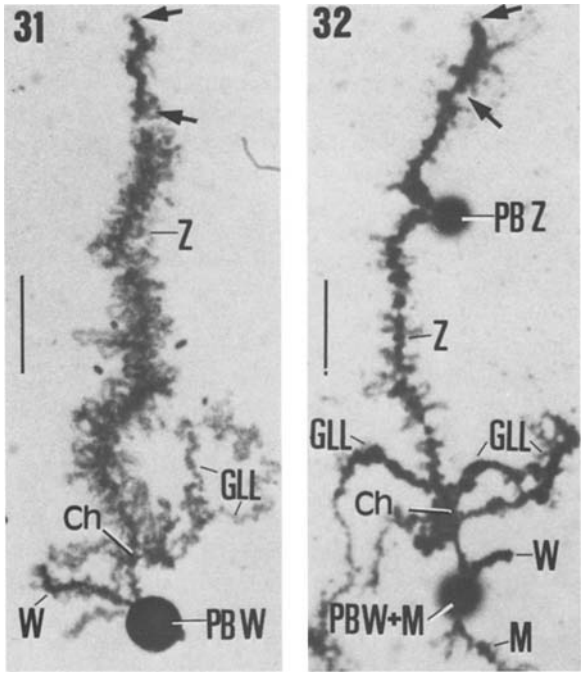
Figures 27–30. Sex bivalents from pigeon with separate (27 and 28) and fused (29 and 30) protein bodies (PB). The two bivalents shown in these figures were first stained with DAPI (27 and 29) and then restained with Coomassie (28 and 30). Z, W, axis of Z- and W-chromosomes; GLL, giant lumpy loops at the telomeres (T) Z and W chromosomes in the region of crossing-over; arrows show the chiasmata; condensed regions on Z-LBCs are marked by arrowheads.

28, 33). When the chiasma has a near telomeric position, only the point of origin of the GLLs provides a clue as to its position (Figure 2).

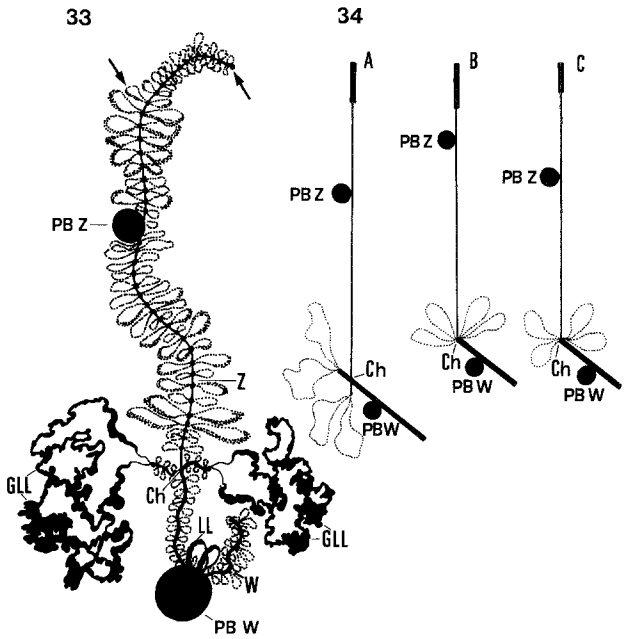
The sex bivalents of sparrow (*Passer domesticus*) and chaffinch (*Fringilla coelebs*)

The ZW bivalents of sparrow and chaffinch are basically similar to that of the pigeon (Figure 34A, B, C). The axis of the Z chromosome consists of typical chromomeres and loops and is in sharp contrast to the condensed and loopless axis of the W. There is one region of uniformly short loops with more condensed chromomeres on the free arm of the Z chromosome. As in the pigeon, there are no GLLs at the free ends of the sex LBCs of chaffinch and sparrow.

LBCs from sparrow and chaffinch, like those from pigeon, have PBs near their centromeres and GLLs at their ends. In chaffinch, the PB occupies a nearly median position on both the W and Z chromosomes (Figures 32 & 34C). In sparrow, the PB of the W chromosome is not median in position but lies more toward the chiasmate end of the chromosome than in chaffinch (Figures 31 & 33B). The PB of the Z chromosome in sparrow is about one-third of the chromosome's length from the free end (Figure 33B). Homologous and heterologous fusions of



Figures 31 & 32. Sex bivalents from sparrow (31) and chaffinch (32) in permanent preparations after staining with Coomassie. Z, W, axes of Z- and W-chromosomes; PB, protein body; note the absence of protein body on sparrow Z-LBC (31): it may have been lost during manual isolation; protein body on chaffinch W chromosome (32) fused with protein body of a microchromosome (M); GLL, giant lumpy loops at the chiasma locus; chiasmata are shown by arrows; condensed regions on Z-LBCs are marked by arrowheads.



Figures 33 & 34. Schematic drawing of pigeon ZW bivalent (33) and diagrams of the basic organization of sex bivalents from pigeon, sparrow and chaffinch (34 a, b, & c). Z, W, axis of Z- and W-chromosomes; PB, protein body; LL, lumpy loops near the PB locus on pigeon W chromosome; GLL, giant lumpy loops near the chiasma locus; chiasmata are shown by arrows; thin lines on 34 a, b, & c signify a normal lampbrushy axis, thick lines signify a condensed axis; condensed region on free end of Z-LBC is marked by arrowheads.

PBs in these species are common (Figure 32) which complicates both the isolation and the identification of the chromosomes.

Discussion

Five lines of evidence demonstrate that the 'asymmetrical macrochromosome' of bird karyotypes is indeed a ZW sex chromosome bivalent: a short condensed segment that is the W and a longer and typically lampbrushy segment that is the Z. First, it carries two protein bodies that occupy the positions that would be expected if, as on the autosomes, they were located near to the centromeres of the proposed Z and W segments. Second, there are two pairs of marker loops at the point where the two structurally distinct segments meet and, since all autosomes terminate in loops of this kind, it seems reasonable to conclude that at the meeting of these segments, the ends of the Z and W lie close to one another. Third, an actual chiasmate association, in the form of a sub-terminal joining and crossing over of axes of the lampbrushy and condensed segments has been observed. Fourth, *in situ* hybridization with a telomere probe has demonstrated three sites of localization of the telomere repeat: two on the ends of the asymmetrical chromosome and one in the 'interstitial' region, at the point where GLLs arise. These data are consistent with the terminal position of a solitary recombination nodule in the short synaptonemal complex formed by the ZW bivalent in chicken and quail (Rahn & Solari 1986, Solari *et al.* 1988, Solari 1992). Lastly, repeat DNA sequences that are specifically and exclusively localized in the chicken W chromosome bind only to the short condensed segment of the lampbrush 'asymmetrical chromosome' under conditions of *in situ* DNA/DNA hybridization. Recently it has been shown by *in situ* hybridization (S. Mizuno, personal communication) that two unique Z-specific genes are located in the lampbrushy part of the asymmetrical chromosome: one, IREBP, is in the region II near the border with region I; another, ZOV3, is in the middle of region III.

It has repeatedly been demonstrated in amphibians and birds (Callan 1986, Chelysheva *et al.* 1990) that the relative lengths and arm ratios of chromosomes in the lampbrush form correspond to those of the same chromosomes at mitotic metaphase or meiotic prophase (pachytene). The bird sex chromosomes that we have examined do not follow this rule. The W chromosome is obviously condensed and shortened in comparison with the Z, which is typically chromomeric and lampbrushy. In chaffinch, for example, the W is less than one-quarter the length of the Z, although at mitotic metaphase the relative lengths of

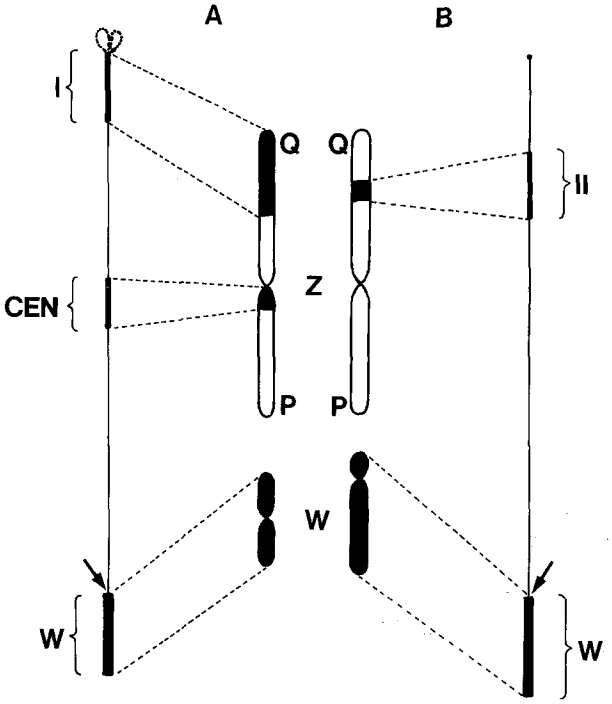


Figure 35. The different morphological regions of the Z and W lampbrush chromosomes and C-banding of mitotic chromosomes from chicken (**a**) and quail (**b**). C-positive heterochromatin blocks on mitotic chromosomes are shown black; thin lines on the LBCs signify a lampbrushy axis; thick lines signify a condensed axis; I and II, condensed region on chicken and quail Z-LBCs respectively; CN, centromere region on chicken Z-LBC. Schemes for mitotic chromosomes after Pollock & Fechheimer (1981); Rodionov *et al.* (1984); de la Sena *et al.* (1991).

these two chromosomes are 8.9 and 11.1 respectively (Ray-Chaudhuri 1973). The W lampbrush chromosomes of quail and turkey show clear signs of hypercondensation in the helical and sometimes clumped conformations of the W chromatin and its intense fluorescence after staining with DAPI. The estimated position of the centromere in the W lampbrush chromosome, as judged from the position of the PB, agrees well with the known position for the corresponding chromosome in metaphase, for example, submetacentric in pigeon (Mayr & Auer 1988, Mayr *et al.* 1990) and subtelocentric in the sparrow and chaffinch (Bulatova *et al.* 1972, Piccini & Stella 1970).

The relative lengths of the Z-LBC and the Z-mitotic chromosome are the same, but their arm ratios differ. It is impossible to measure arm ratios of Z-LBC in turkey and quail, but, if the PB is a reliable marker for the approximate position of the centromere, then in pigeon, sparrow and chaffinch the two arms of the Z-LBCs are obviously of different lengths (Figure 34A, B, C), whereas mitotic-Z chromosomes from these species are decidedly metacentric. The chicken mitotic Z chromosome is nearly metacentric (centromere index 0.49) and the single recombination nodule has been shown to be situated on its shorter arm (Solari *et al.* 1988, Solari 1992). However, according to our own observations on chicken, the clearly submetacentric Z-LBC (centromere index 0.45) has a chiasma in its long arm (Figure 19D). We consider that these differences in arm ratios result from a non-uniform pattern of condensation along the axis of the Z-LBC. The sub-telomeric portion of the free arm of the Z-LBC in chicken (region I), pigeon, sparrow and chaffinch is always occupied by large, brightly DAPI-positive and closely packed chromomeres with short loops, signifying a greater degree of general condensation of chromatin than in other parts of the LBC.

In general, our observations suggest a correspondence in birds between blocks of C-band heterochromatin in mitotic metaphase chromosomes and relatively condensed regions with short loops or no loops in LBCs (Rodionov *et al.* 1989). It is known that the W chromosome in at least four of the species included in this study consists almost entirely of Giemsa C+ material (Pollock & Fechheimer 1981, Rodionov *et al.* 1984, Auer *et al.* 1987, Mayr & Auer 1988). C banding of the Z chromosome has been described in detail only in the chicken and quail. In the chicken, a large heterochromatic block occupies a terminal portion of the longer arm of the Z (Pollock & Fechheimer 1981, Rodionov *et al.* 1984) and this would seem to correspond to region I of the Z-LBC. Quail has a smaller interstitial C-band (de la Sena *et al.* 1991) which may correspond to region II of its Z-LBC. The main features of correspondence between mitotic and lampbrush Z and W chromosomes in chicken and quail are shown in Figure 35. The Z-LBCs of pigeon, sparrow and chaffinch all show quite clear linear differentiation into regions with uniformly short or longer loops, coarse or fine chromomeric arrangements. In this regard, it would be interesting to examine the Giemsa C-band patterns on the mitotic chromosomes of these species.

In quail and turkey, the W chromosome is of medium size in relation to the rest of the karyotype and in its lampbrush form it is strongly condensed and totally lacking in discernible chromomeric organization. In chicken, on the other hand, the W is a microchromosome and in its lampbrush form it consists of a constant number of distinct chromomeres, each one individually recognizable on the basis of relative size, shape, position and brightness after staining with DAPI fluorochrome (Figure 19E, G). Between 70 and 90% of the entire DNA of the chicken W chromosome is accounted for by just two repeat sequences, the *Xho*I family (Tone *et al.* 1984, Kodama *et al.* 1987) and the *Eco*RI family (Saitoh *et al.* 1991). Experiments involving pulse field electrophoresis have shown that these two sequence families occupy different loci on the W chromosome (Saitoh *et al.* 1991). The *Xho*I family was detected by *in situ* hybridization in the pericentromeric region of the mitotic W chromosome. The *Eco*RI family was detected at two loci: the major one extended over most of one entire arm of the chromosome; the minor one formed a band in the proximal half of the other arm (Saitoh & Mizuno 1992).

In our *in situ* hybrids, each of the repeat sequence families was detected at a separate locus, near the middle

of the chromosome, in the third chromomere for the *Xho*I family and at the extreme end of the W-LBC in the free terminal chromomere 1 for the *Eco*RI family (Figure 19F). Both are the brightest of the chromomeres after staining with DAPI. Now that we know that the different W-specific repeats are exclusively localized in certain chromomeres, it may be useful to investigate W-chromomere organization in chickens and breeds of chicken that have a low number of copies of the *Xho*I or the *Eco*RI repeats (Tone *et al.* 1984, Saitoh *et al.* 1991). Conversely it should be interesting to look for the *Eco*RI repeat sequence family in chickens that lack the terminal chromomere on the W.

All the LBCs of all the species that we have examined, except quail, have distinctive loops at their extreme ends. These terminal loops may be quite small, as is often the case at the free end of the Z and W chromosomes, or they can be spectacularly large, as in the case of the GLLs at the chiasmate ends of both sex chromosomes. That these loops are actually situated at the very end of the chromosome may be of special interest to persons concerned with fundamental lampbrush structure and function. Where these terminal loops hang free from the end of the chromosome, as seen in Figures 19, 26, 33, we must conclude that the chromosome actually ends at the end of a very long transcription unit or a tandem series of transcription units, and not at a telomeric granule or chromomere as has hitherto been supposed on the basis of detailed morphological studies of the lampbrush chromosomes of amphibians (Callan 1986).

The existence of large free-hanging loops that terminate with a small cluster of telomeric DNA sequences can be seen on a grand and altogether spectacular scale in the germinal vesicles of woodpigeon (*Columba palumbus*) and this will be the subject of our next communication (Solovei & Macgregor in preparation).

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References

Auer H, Mayr B, Lambron M, Schleger W (1987) An extended chicken karyotype, including the NOR chromosome. *Cytogenet Cell Genet* **45**: 218–221.

Bulatova NS, Radjabli SI, Panov EN (1972) Karyological description of three species of the genus *Passer*. *Experientia* **28**: 1369–1371.

Chelysheva LA, Solovei IV, Rodionov AV, Yakovlev AF, Gaginskaya ER (1990) The lampbrush chromosomes of the chicken: the cytological maps of macrobivalents. *Cytology* **32**: 303–316.

Callan HG (1986) *Lampbrush Chromosomes*. Berlin: Springer Verlag.

Callan HG, Gall JG, Berg CA (1987) The lampbrush chromosomes of *Xenopus laevis*: preparation, identification, and distribution of 5S DNA sequences. *Chromosoma* **95**: 236–250.

de la Sena CA, Fechheimer NS, Nestor KE (1991) Variability of C-banding patterns in Japanese quail chromosomes. *Genome* **34**, 993–997.

Gaginskaya ER (1972) The nuclear structures of oocytes in adult birds. II. Protein bodies and the karyosphere. *Cytology* **14**: 568–578.

Gaginskaya ER, Kropotova EV, Tsvetkov AG, Vengerov YuYu (1984) Morphology of transcription in lampbrush chromosomes from avian oocytes. *Cytology* **26**: 1064–1065.

Gall JG (1954) Lampbrush chromosomes from oocyte nuclei of the newt. *J Morphol* **94**: 283–352.

Gall JG, Stephenson EC, Erba HP, Diaz MO, Barsacchi-Pilone G (1981) Histone genes are located at the sphere loci of newt lampbrush chromosomes. *Chromosoma* **84**: 159–171.

Hutchison NJ (1987) Lampbrush chromosomes of the chicken *Gallus domesticus*. *J. Cell Biol* **105**: 1493–1500.

Khutinaeva MA, Kropotova EV, Gaginskaya ER (1989) Morpho-functional organisation peculiarities of the lampbrush chromosomes of oocytes in the pigeon, *Columba livia*. *Cytology* **31**: 1185–1193.

Kodama H, Saitoh H, Tone M, Kuhara S, Sakaki Y, Mizuno S (1987) Nucleotide sequences and unusual electrophoretic behavior of the W chromosome-specific repeating DNA units of the domestic fowl, *Gallus gallus domesticus*. *Chromosoma* **96**: 18–25.

Kropotova EV, Gaginskaya ER (1984) Lampbrush chromosomes from the Japanese quail oocytes: light and electron microscopic study. *Cytology* **26**: 1008–1015.

Macgregor HC (1987) Lampbrush chromosomes. *J Cell Sci* **488**: 7–9.

Macgregor HC, Varley JM (1988) *Working with Animal Chromosomes*. 2nd ed. Chichester: John Wiley and Sons.

Mayr B, Auer H (1988) A method to induce Giemsa staining resistance of avian W chromosomes: detection of an additional W chromosome in turkey. *Genome* **30**: 395–398.

Mayr B, Lambrou M, Kalat M, Schleger W, Bigelbach A (1990) Characterization of heterochromatin by sequential counterstain-enhanced fluorescence in three domestic bird species: *Meleagris gallopavo*, *Columba livia domestica*, and *Anser anser* L. *J Heredity* **81**: 468–475.

Moyzis RK, Buckingham JM, Cram LS *et al.* (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* **85**, 6622–6626.

Ohno S (1961) Sex chromosomes and microchromosomes of *Gallus domesticus*. *Chromosoma* **11**: 484–498.

Piccini E, Stella V (1970) Some avian kariograms. *Caryologia* **23**: 189–202.

Pollock B, Fechheimer NS (1981) Variable C-banding patterns and proposed C-band karyotype in *Gallus domesticus*. *Genetica* **54**: 237–279.

Rahn MI, Solari AJ (1986) Recombination nodules in the oocytes of the chicken, *Gallus domesticus*. *Cytogenet Cell Genet* **43**: 187–193.

Ray-Chaudhuri R (1973) Cytotaxonomy and chromosome evolution in birds. In: Chiarelli AB, Capanna E, eds. *Cytotaxonomy and Vertebrate Evolution*. London: Academic Press, pp 425–481.

Rodionov AV, Dukelskaya AV, Chelysheva LA, Smirnov AF, Yakovlev AF (1984) Molecular heterogeneity of the domestic fowl (*Gallus domesticus*) heterochromatin. *3rd National Conference on Cytogenetics, Plovdiv*, Poland. pp. 138–141.

Rodionov AV, Chelysheva LA, Kropotova EV, Gaginskaya ER (1989) Heterochromatic regions of the chromosomes in mitosis and at the lampbrush stage in the domestic fowl and Japanese quail. *Cytology* **31**: 867–873.

Saitoh Y, Saitoh H, Ohtomo K, Mizuno S (1991) Occupancy of the majority of DNA in the chicken W chromosome by bent-repetitive sequences. *Chromosoma* **101**: 32–40.

Saitoh Y, Muzuno S (1992) Distributions of *Xho*I and *Eco*RI family repetitive DNA sequences into separate domains in the chicken W chromosome. *Chromosoma* **101**: 474–477.

Solari AJ (1977) Ultrastructure of the synaptic autosomes and ZW bivalent in chicken oocytes. *Chromosoma* **64**: 155–165.

Solari AJ (1992) Equalization of Z and W axes in chicken and quail oocytes. *Cytogenet Cell Genet* **59**: 52–56.

Solari AJ, Fechheimer NS (1988) Quadrivalent formation in a tetraploid chicken oocyte. *Genome* **30**: 900–902.

Solari AJ, Fechheimer NS, Bitgood JJ (1988) Pairing of ZW gonosomes and the localized recombination nodule in two Z-autosome translocations in *Gallus domesticus*. *Cytogenet Cell Genet* **48**: 130–136.

Solovei IV, Khutinaeva MA, Chelysheva LA, Rodionov AV, Gaginskaya ER (1990) Lampbrush sex bivalent of birds: identification and structure. *Cytology* **32**: 817–825.

Solovei IV, Gaginskaya ER, Allen T, Macgregor H (1992) A novel structure associated with a lampbrush chromosome in the chicken, *Gallus domesticus*. *J Cell Sci* **101**: 759–772.

Tsvetkov AG, Gaginskaya ER (1983) The nuclear matrix of oocytes of the chaffinch (*Fringilla coelebs* L.) *Cytology* **25**: 649–654.

Tone M, Sakaki Y, Hashiguchi T, Mizuno S (1984) Genus specificity and extensive methylation of the W chromosome-specific repetitive DNA sequences from the domestic fowl. *Gallus gallus domesticus*. *Chromosoma* **89**: 228–237.