THE CHROMOSOMES OF THE DOMESTIC CHICKEN[1].

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(With Plates XXVII–XXX.)

Introduction.

The chromosomes of vertebrates—particularly those of birds and mammals—have proved infinitely more difficult to study than those of invertebrates. Not only are the chromosomes small and very numerous, but they show a marked tendency to "clump" in the metaphase plate if fixation is delayed more than a few seconds after the death of the animal. So important is instantaneity of fixation that it is quite useless to attempt to fix whole embryos or pieces of testis in any of the ordinary fixatives such as Bouin or its modifications, since the liquid will take far too long to penetrate to the interior of such a piece of tissue.

It is only since about 1920 that this necessity for very rapid fixation has been realised; thus papers on vertebrate chromosomes written before that date are (with one or two exceptions) of no value save as a warning to future cytologists. The literature on the chicken is very unsatisfactory and contradictory; the only worker who has studied the whole chromosome cycle is Guyer (1916), whose work was performed on very badly fixed material, although possibly slightly better than that used by Boring and Pearl (1914). Several more recent papers are incomplete in that their authors only studied the somatic chromosomes in embryonic material, neglecting the spermatogenesis.

It was in view of the fact that modern methods involving very rapid fixation had led to apparently successful results on the pigeon, duck and turkey (Oguma, 1927 and Werner, 1927 and 1931) that I decided to reinvestigate the whole cycle of the chicken.

To Prof. D. M. S. Watson, F.R.S., I am indebted for really helpful advice on the technical problems of high-power microscopy. Mr Tom Newman, Secretary of the Scientific Poultry Breeders' Association, very kindly supplied me with much of the material for the work.

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Methods.

All the material used in this work was fixed in Allen's Chromic Bouin with urea at 38° C. and stained in iron alum haematoxylin. No special precautions were employed during dehydration, as experience showed that the elaborate methods used by Werner (1927) were quite superfluous.

For the study of somatic chromosomes I used 40-hour embryos which were rapidly removed from the yolk, dropped into a vial of the warm fixative and cut into several pieces. These embryos were afterwards sectioned at 10 μ. For the spermatogenesis I used smears of adult testes, since I found it quite impossible to get good penetration of even very small *fragments*. In the case of a monocellular layer on a slide, however, the conditions are ideal for instantaneous fixation, and I believe that I have in this way succeeded in altogether eliminating the tendency to "clump."

The drawings were done with a Zeiss 2 mm. apochr. objective and a No. 18 Compensating Ocular. I found that a Watson Holoscopic Oil Immersion Condenser greatly improved the resolution of the finer detail.

The somatic chromosomes.

Mitoses are very numerous in 40-hour embryos, but only a small percentage were suitable for detailed study; some of the best of these are shown on Plates XXVII and XXVIII. The first thing which strikes one on looking at these somatic metaphase plates is the great range in size of the individual elements; the larger ones at the periphery of the plate are of quite respectable dimensions, but the smallest ones in the centre are absolutely on the limit of the resolving power of the microscope (about 0·2 μ in diameter). This great range in size of the chromosomes appears to be characteristic of birds in general and is quite unparalleled elsewhere. In some reptiles one finds very small and quite large chromosomes in the same metaphase plate, but in these there is a sharp division into "macro-" and "micro-chromosomes" with no intermediates, whereas in birds all intermediates are present so that there is a complete gradation between the largest and the smallest.

It is unfortunately not possible to determine the sex of 40-hour embryos, but those that I have studied fall into two classes: (1) those in which there are four long J-shaped chromosomes with sub-median spindle attachments and (2) those in which only three such elements can be recognised. It is clear that in the first case we are dealing with two pairs of chromosomes which are nearly equal in length, and that

in the second case one member of one pair is absent. Since female heterogamety has been conclusively proved by genetical means one must conclude that the second class are female embryos, the first class males. The two pairs of chromosomes in question are so nearly equal in size that it is extremely difficult to decide whether it is one of the longest pair or one of the next longest that is missing in the female, but I am inclined to believe that it is the former and have labelled the figures on this assumption.

In some of the division figures several chromosomes are connected together by more or less lightly staining threads. These threads have been seen by many other workers on vertebrate chromosomes and different interpretations have been placed upon them. I consider that there can be no doubt that they are artefacts which are a preliminary to clumping and I have hence rejected all cells in which there were many such threads as unfit for detailed study. In many of the best metaphases they are altogether absent.

All really well-fixed metaphase plates which are favourably oriented and in which the chromosomes do not overlap too much show over 60 chromosomes. In the case of the male cells 66 is the highest number recorded, while in the female cells I was never able to count more than 65. I am inclined to consider that these are the true diploid numbers in the two sexes of the chicken. Cells in which only 63 or 64 chromosomes are visible are frequent but it seems probable that in these one or two of the smallest chromosomes are lying so close to other chromosomes as to appear invisible as separate elements. Cells in which less than 60 chromosomes can be recognised are clearly badly fixed and I have no doubt that a varying amount of "clumping" has taken place in them.

In the course of my work I have seen nothing which suggested the presence of a *W*-chromosome in the female, but in view of the very large chromosome number it is quite possible that one or two of the smaller elements may function as a mate to the unpaired *Z*-chromosome during the reduction division.

THE SPERMATOGENESIS.

Spermatogonia are very rare in my smears, since, as already noticed by Guyer (1916), they seem to adhere very closely to the walls of the tubules so that they do not readily spill out on to the slide. I only found three or four of them and these were not suitable for detailed study. They resembled male somatic plates, except that the longer chromosomes were shorter and thicker.

The haploid chromosomes were mainly studied in diakinesis. This is a particularly favourable stage for observing them, since they are spread all over the interior of the nuclear membrane instead of being crowded close together in one place as at metaphase. In all late diakineses 33 chromosomes were visible; the earlier stages are more difficult to study since the chromosomes are so long and straggling, but here again the number was always 33 plus or minus 1.

The great range in size of the chromosomes is again obvious at diakinesis; the larger ones are clearly bivalent, but in the smaller ones no detail can as a rule be made out. The largest bivalent represents the sex-chromosomes seen in the somatic cells.

In the first spermatocyte metaphase 33 chromosomes can again be counted (Pl. XXX, Figs. 19–22), the larger ones at the periphery, the smaller ones in the centre. At anaphase the larger ones lag on the spindle but all of them eventually divide (Pl. XXX, Figs. 23 and 24). In side views of first spermatocyte metaphases a chromosome is sometimes displaced towards one pole in the process of smearing so that it appears to be passing undivided to that pole; I would suggest that it was appearances such as this that led Guyer to the belief that the sex-chromosome passed undivided to one pole at the first spermatocyte division.

All the second spermatocyte divisions in my material were unfortunately "clumped" to a greater or less extent, but it is fairly obvious from the best of them that no secondary pairing or association of chromosomes as described by Guyer takes place.

The correspondence between the cytological and the genetical evidence.

The cytological features reported above are in complete agreement with all the salient features of avian genetics, viz. female heterogamety, male homogamety, very little linkage and the fact that the majority of linked genes which have been reported are sex linked.

Criticism of literature.

To deal with papers published before 1920 would be a mere waste of time; there remain, however, several more recent papers to be discussed. In his very careful and thorough work on the pigeon Oguma (1927) obtained results which are in every way identical with my own except that this bird appears to have a diploid number of 62 instead of 66.

Werner (1927 and 1931) has in a couple of papers reported that the diploid number of the duck and turkey is 76 in the male and 77 in the

female. I have no doubt that these numbers are approximately correct, but can they be considered as *exactly* so? If not there is no reason to assume the existence of two *W*-chromosomes and thus the duck and the turkey fall into line with the other birds (chicken and pigeon) recently studied. While accepting Werner's results as being at any rate substantially correct, I am not prepared to accept the very complicated scheme of sex-chromosomes which she puts forward.

Three papers—those of Akkeringa (1927), Hance (1926) and Shivago (1924)—can be dealt with together, since these workers all came to similar conclusions. They studied somatic chromosomes without making any adequate study of the spermatogenesis and came to the conclusion that the diploid number was between 30 and 40. There can be no doubt that they were dealing with material which, although superior to that used by Guyer, was nevertheless not free from "clumping," so that the smaller chromosomes in the centre of the plates appeared less numerous than they should have done. Had these workers studied the haploid number in diakinesis or first spermatocyte metaphase they would probably have been led to doubt their conclusions on the subject of the diploid number.

SUMMARY.

(1) The diploid number in the chicken is 66 plus or minus two.

(2) The haploid number is 33 plus or minus one.

(3) There is a very great difference in size between the largest and the smallest chromosomes with every gradation in between.

(4) The largest pair of chromosomes in the male is represented by a single element in the female. These are regarded as sex-chromosomes.

(5) No sign of a *W*-chromosome was observed.

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EXPLANATION OF PLATES XXVII—XXX.

PLATE XXVII.

Female somatic chromosomes.

Fig. 1. Very late prophase; probably 64 chromosomes visible.
Fig. 2. The same; 62 or 63 chromosomes visible.
Fig. 3. Metaphase; 65 chromosomes.
Fig. 4. Only 61 chromosomes apparent.
Fig. 5. 64 chromosomes.
Fig. 6. 65 chromosomes visible.

PLATE XXVIII.

Male somatic chromosomes.

Fig. 7. 66 chromosomes.
Fig. 8. 66 chromosomes.
Fig. 9. 65 chromosomes visible.
Fig. 10. Only 62 chromosomes can be seen.
Fig. 11. 66 chromosomes.
Fig. 12. Apparently only 62 chromosomes.

PLATE XXIX.

Figs. 13–18. Diakineses, all showing 33 chromosomes.

PLATE XXX.

Figs. 19–22. First spermatocyte metaphases showing 33 chromosomes.
Figs. 23 and 24. First spermatocyte anaphases showing lagging of the larger chromosomes.

Explanation of lettering.

In all the figures *Z* is a sex-chromosome, *Zz* a sex-bivalent. *B* and *b* are the largest pair of autosomes.