The Meiotic Chromosome of the Fowl

Earl H. Newcomer

Department of Botany, Univ. of Conn., Storrs, Conn., U.S.A.

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Introduction

A review of the literature concerning the chromosomes of the domestic fowl and avian cytology in general reveals widely divergent points of view. Matthey (1949) considers the chromosomes of the fowl to be the least known among the vertebrates, whereas Yamashina (1944), reported constancy in number, morphology and precision in division of all seventeen races investigated by him. Most investigators have been content to estimate the chromosome number or have reported them as uncountable (van Brink and Ubbels 1956). Painter and Cole (1943) have described the special difficulties of avian cytology and White (1949) has recently stated, "Thus the student of avian speciation must, for the present, resign himself to the fact that he cannot use the method of comparative cytology, which has proved so valuable in other groups, simply because the technical obstacles do not permit us to arrive at an absolutely accurate morphological description of the chromosome set of any species of bird (the position as regards the mammals and reptiles is substantially better, but not entirely satisfactory even yet). Strategic considerations should cause serious investigators to avoid vertebrate material for chromosome work in the future, except in special cases."

The number of chromosomes in the fowl has been reported from the extremes of 'more than six' by Miss Loyez in 1906 to the recent high of eighty-two (van Brink and Ubbels 1956) with a mode around forty. The tendency has been to accept the higher numbers as being the most accurate and Yamashina (1944) reported the precise counts of seventy-seven in the female and seventy-eight in the male. The charge of technical inadequacy has been widely used as an explanation for the divergent results. The confusion seems to be due partly to technique, but in as large a measure to the limitations of most investigations to metaphase plates and the failure to follow the chromosomes throughout the meiotic and mitotic cycles. Thus, while general agreement has long been reached regarding the identity of the six pairs of so-called macrochromosomes, the work of Guyer (1916), Hance (1942, 1926a, b, c), Painter and Cole (1943), Sokolow and Trofinow (1933) and Crew (1933), has clearly shown the necessity for further study of the so-called microchromosomes because of their fluctuations in number, size and general behavior.

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In our report of the spermatogenesis of the domestic fowl (Newcomer and Brant 1954), we relegated the micro-chromosomes to the category of accessory or supernumerary chromosomes on the basis of structure, fluctuation in number, allocyclic behavior and their disappearance during the maturation of sex cells. In a later paper on the mitotic chromosomes (Newcomer 1957), these have been removed from chromosomal status and designated as chromosomoids, which apparently function as adjuvants to the nucleic acid cycle of the chromosomes in addition to the usual functions ascribed to heterochromatin in the nucleus.

A similar uncertainty has existed as to the identity of the sex chromosome. Prior to the genetic determination of the female as being heterozygous for sex, Guyer (1921), in an otherwise excellent study, reported the largest chromosome of the male as being unpaired and subsequent workers, while accepting the sex determining role of the female, have variously identified the first, second and fifth largest chromosomes with sex determination because presumably unpaired. But following the work of Suzuki (1930) and Unger (1936), the metacentric, fifth largest chromosome is now generally accepted as being unpaired in the female and therefore associated with sex determination. There still remains the problem, however, of reconciling the cytogenetic discrepancy between the high cross over values of the sex linkage data (Warren 1949) with the fifth largest metacentric chromosome and its low chiasma frequency (Newcomer and Brant 1954).

This paper is concerned with the meiotic chromosome cycle and presents additional corroborative evidence for the non-chromosomal status of these nuclear constituents which have been previously referred to as micro- or accessory chromosomes and which we have described as chromosomoids (Newcomer 1957).

Methods and materials

For this study, the gonads of sexually mature males of the breeds Rhode Island Red and Columbian were used after comparative studies showed no discernible differences in their chromosomes.

The technical difficulties of securing well-spread metaphase plates of avian chromosomes is well known and for this reason a number of pre-treatments of freshly excised tissues prior to fixation were used to spread the chromosomes. Successful techniques consisted of placing sliced tissues in half strength Gey's nutrient solution to which was added either 0.1 % versene, 0.02-0.5 % colchicine or coumarin or paradichlorobenzene to saturation and incubating for two to four hours. In addition, tissues were pre-treated with distilled water at room temperature for a period of ten minutes as recommended by Makino and Nishimura (1952). Tissues were also processed without pre-treatment. All tissues were fixed in Newcomer's fluid (Newcomer 1953), smeared and stained with either Feulgen or propionic

carmine. Gonads were also processed by the paraffin method, sectioned and stained with Feulgen, haematoxylin or crystal violet. The advantages of the smear technique are so obvious that the use of a sectioning technique is not recommended for avian chromosomes.

Observations

The result of this partial re-survey of the meiotic cycle with the aid of tissue pre-treatment techniques adds convincing evidence for the non-chromosomal status of the chromosomoids and their functioning as adjuvants in the nucleic acid cycle of the chromosomes. They appear to originate from chromocentric heterochromatin in a manner similar to that previously described for the mitotic cycle (Newcomer 1957). Pre-prophase primary spermatocyte nuclei are characterized by the appearance of Feulgen positive chromocentric heterochromatin and either the nucleoli are also Feulgen positive at this time or are surrounded by closely appressed chromatin which renders them apparently Feulgen positive (fig. 1). At zygotene and pachytene, the chromocenters have developed into conspicuous, irregular masses intimately associated with the developing chromosomes (fig. 12). As prophase proceeds, the heterochromatic masses partially disintegrate into smaller discrete units, some of which seem to be incorporated into the chromosomes while the rest may be scattered about the nucleus in various conditions suggesting both fragmentation and fusion (fig. 2). Great variations in numbers and size of the chromosoids can be seen (cf. figs. 2, 3, 8, 9). In size they range from chromosomal to the limits of visibility.

At diplotene and diakinesis, they show evidences of ectopic pairing, fusion and fragmentation (fig. 2, 3). At metaphase, they are variably heteropycnotic (fig. 4) and are often associated with each other and the chromosomes by an anastomosing complex of inter-chromosomal strands (figs. 8, 9). In lateral views of metaphase or early anaphase, though aligned with the chromosomes, they present no evidences of the possession of centromeres, but segregate as polycentric or acentric units (fig. 10). They may either segregate precociously or lag in disjunction and in either event, their distribution to the daughter nuclei is only roughly quantitative.

With the reductional disjunction, there is a marked reduction in number and volume of the chromosomoids and by second metaphase (fig. 5) and anaphase (fig. 11), they have virtually disappeared, as was described in our previous study (Newcomer and Brant 1954). It is interesting in retrospect to recall that Guyer (1916) and Levine (1931) also observed this reduction in the number of elements in the sex cells of the fowl. They reported a reduction of the eight or nine bivalents of the first metaphase to four or five chromosomes at the second anaphase and this was thought by Guyer to be the result of the fusion of chromosomes. We have ascribed the pheno-

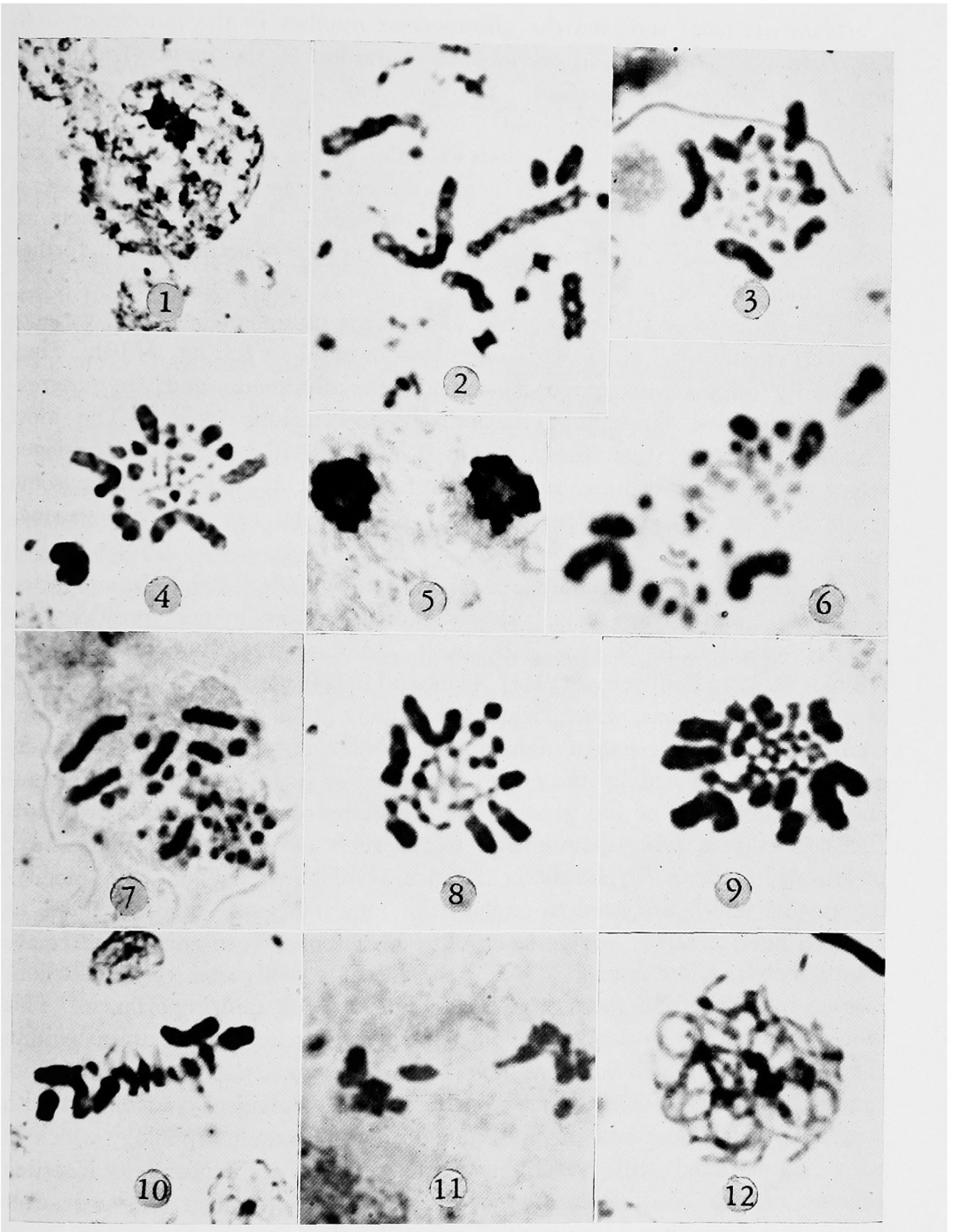
menon to the probable utilization of the chromosomoids in the meiotic DNA cycle. In any event and whatever their function, they can hardly be considered as chromosomes.

Discussion

The addition of chromosomoids to the categories of non-euchromosomal elements in the nucleus such as accessory, inert, supernumerary, B chromosomes; the diminuted or eliminated chromosomes of the Ascaridae, the beetle Distycus and some of the Lepidoptera; the limited chromosomes of Sciara and the so-called E chromosomes of the gall midges constitutes an interesting extension of the spectrum of sub-chromosomal constituents which span the extremes from hetero- to eu-chromaticity. Whether these bodies are in any degree homologous, what they represent in the evolution of genetic systems and whether they are incipient or derivative in origin is still a matter of speculation. Their persistence might suggest that they confer a selective advantage and it is possible that some of them contribute an essential function to the chromosome cycle and the genom of the organism. Cytologic and genetic data may suggest such a dual function for the chromosomoids in the fowl.

The heterochromatic origin, fluctuation in numbers and behavior of the chromosomoids throughout the mitotic and meiotic cycles and especially their partial to complete disappearance during mitosis and meiosis, when intracellular syntheses appear to be suspended (Pollister 1952) suggests that they constitute a reserve supply of nucleic acids for chromosome replication. The function of heterochromatin in the reproductive cycle of the chromosomes has been attested by Schultz (1932), Callan (1942) and Pontecorvo (1944). The numerical fluctuations of the heterochromatic chromosomoids may thus be a reflection of the division index and rate of chromosomal replication.

It seems clear from a review of the literature and the present study that the confusion concerning the chromosome number of the domestic fowl and probably birds in general is due to fundamental differences in the kind of nuclear constituents involved and is not merely a matter of technique. The presumed dicta of cytogenetics have been interpreted too literally and the demanded constancy in number and morphology of the chromosomes of a species have included elements which, though of undoubted similar chemical properties, are not chromosomal at all. In our previous study (Newcomer and Brant 1954) we shared the natural reluctance of previous investigators to question the possibility that the so-called microchromosomes might be other than chromosomal in satatus and described them as accessory or supernumerary chromosomes. Accessory chromosomes are defined as "... such extra chromosomes as are not homologous with those of the ordinary complement and whose number is in equilibrium in a population" (Melander 1950).



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Hance (1926a) reported the chromosome number in the mitotic cycle to vary between 35 and 70 and ascribed the variation to the pseudo-fragmentation of the micro-chromosomes. Sokolow and Trofimow (1933) suggested polysomy and fragmentation and Crew (1933), observing their precocious separation, believed them to be non-essential to the species, resulting from fragmentation and fusion. Such behavior and numerical fluctuation seems anomalous for even supernumerary chromosomes. In addition to their inconstancy in number and behavior is their acentric structure which further belies their chromosomal status.

The chromosomoids are acentric, allocyclic in origin and behavior, variably heterochromatic, and show wide variations in numbers (cf. fig. 9, 10). They apparently originate from chromocentric heterochromatin and are progressively diminished throughout the mitotic and meiotic cycles. The most plausible theory for their function which is supported by cytologic evidence seems that they constitute reserves of nucleic acids for the chromosome synthesis cycle and are utilized as such. That they are not chromosomes seems clear.

A genetic effect or function of the chromosomoids seems to be supported by breeding and linkage data. Heterochromatin is no longer considered by most investigators to be genetically inert. Mather (1944) and others (Goldschmidt (1948), Pontecorvo (1944), Hannah (1951)) consider heterochromatin as carrying polygenes, which, while individually not essential to the organism, contribute in the aggregate a modifying and balancing effect upon the major, or oligogenes, carried by the euchromatin and as such, constitute an integral and important part of the genom. The genetics of the domestic fowl (Hutt 1949) is replete with quantitative terms such as incomplete dominance, pleiotropy, epistasis, hypotasis, penetrance, complementary genes and modifying factors which are used to explain the inheritance of such characters as crooked keel, creeper, wingless, crooked breastbone, rose comb, pea comb, walnut comb, white ear lobes, blue buff, as well as body size, egg production, disease resistance and most other characters of economic importance. This would suggest the presence of some modifying genetic mechanism within the fowl genom which might well be the chromosomoids. Linkage data from the fowl also reveal discrepancies which can be cytologically correlated with variations in somatic and possibly meiotic chromosome morphology which in turn is associated with variations in length of heterochromatic insertion regions of the chromosomes. Such changes would affect the cross-over values and possibly produce position effects by changing the linear proportions of the chromosomes (Cooper 1956). Both Slizynski (1943–47) and Goldschmidt (1948) have observed the tendency to non-specific or ectopic pairing of heterochromatin forming irregular chains as shown in the meiotic chromosomoids (figs. 8, 9). This property shared by the segments of interstitial heterochromatin within the chromosomes, may be responsible for the multivalent

association and occasional appearances of structural hybridity as reported in our previous study (Newcomer and Brant 1954).

The reduction in the chromosome number of the fowl from upwards of eighty to six pairs, if confirmed by further studies, suggests the possibility of a definitive cytogenetics of the fowl. The six haploid chromosomes reported here correspond with the six known linkage groups and our recent finding of a reciprocal translocation involving chromosomes 1 and 2 of the fowl makes possible the association of linkage groups with specific chromosomes (Newcomer, 1959). With further technical improvements such as tissue pre-treatments prior to fixation, the cytology of the fowl may lose some of its onus and the present chasm between its cytology and genetics may be bridged.

Summary and conclusions

Additional evidence is presented for the removal of the so-called microchromosomes from a chromosomal status to that of chromosomoids. The functions of the chromosomoids may be dual. Their disappearance throughout the mitotic and meiotic cycles suggests their utilization in the nucleic acid cycle. Their heterochromatic nature and apparent partial incorporation in chromosomes as interstitial heterochromatin may constitute a quantitative, polygenic effect to the genom as well as affecting linkage relations.

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Explanation of Plate XVI

Figs. 1–12. 1, pre-prophase of primary spermatocyte-Feulgen stain. 2 and 3, late diplotene and diakinesis after pre-treatment by incubation for four hours in half-strength Gey's nutrient solution saturated with paradichlorobenzene. 4, first metaphase—pre-treatment as in fig. 2 and 3. 5, second metaphase after 2 hr. pre-treatment in 0.1 % colchicine in Gey's solution. 6, late diakinesis—pre-treatment as in figs. 2 and 3. 7, first metaphase—no pre-treatment. 8, first metaphase—incubated 4 hrs. in 0.1 % colchicine and half-strength Gey's solution. 9, first metaphase—incubated in half-strength Gay's solution for 4 hrs. 10, lateral view of first metaphase—no pre-treatment. 11, second anaphase—no pre-treatment. 12, pachytene—pre-treated with distilled water for 10 minutes prior to fixation.

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