THE ARRANGEMENT OF CHROMOSOMES IN THE MATURE SPERM OF THE GRASSHOPPER

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INTRODUCTION

The mature sperms of many insects, including the grasshopper (Orthoptera), are very much elongated, needle-like structures. The sperm nucleus is likewise extremely elongated with a diameter as small as or smaller than that of a late prophase or metaphase chromatid. The nucleus tapers to a point at each end, but the anterior end is blunt compared to the posterior end. The most likely arrangement of chromosomes would be either a parallel arrangement in a bundle or a tandem alignment with each chromosome occupying a short segment of the elongated head. Autoradiographic evidence indicates that the latter arrangement exists, and, furthermore, that the chromosomes are randomly disposed along the length of the nucleus in the mature sperms of the grasshopper.

MATERIALS AND METHODS

Males of the grasshopper *Romalea microptera* (Beauvoir) were purchased as nymphs from the Carolina Biological Supply Co., Elon College, North Carolina, during the month of May. The males were injected during the last instar with 20 μc of H^3-thymidine (sp. act. 1000 mc/mM) purchased from the New England Nuclear Corp., Boston. Mature sperms labeled with tritium were obtained within about 60 days after injection with H^3-thymidine when the grasshoppers were kept in the laboratory at 21 to 23°C with 12 hours of light per day. Testes were

fixed in acetic acid–ethanol (1:3) and the squashes were prepared after hydrolysis and staining by the Feulgen reaction.

The coverglasses were removed after freezing the preparations on dry ice (solid CO_2), and, after the slides were rinsed in acetic acid–ethanol (1:3), they were transferred to 70 per cent ethanol and later coated with autoradiographic film (Kodak AR-10). Slides were developed after 6 weeks and prepared for microscopic examination by the procedures previously described (Taylor, 1960).

RESULTS

Under the conditions of culture in the laboratory, labeled chromosomes appear in meiotic division stages in about 23 to 24 days after injection with H^3-thymidine.

Most of the cysts of spermatocytes are unlabeled after one or two closely spaced injections of H^3-thymidine. However, when cysts become labeled, all of the cells of a cyst have labeled chromosomes, and these may usually be classified into one of three characteristic patterns. (1) Some cysts have cells with all chromosomes labeled along most of their length. (2) Other cysts have cells with most of the tritium in the single X chromosome and a few grains localized over regions near the centromeres in the large autosomes. In some of these cells, one of the autosomes of intermediate size is frequently labeled more than the other autosomes, but it has fewer grains per unit length than the X. (3) The third class of cells has all of the chromosomes labeled except the X chromosome. However, the amount of label per unit length varies in the autosomes, with some having short segments which are apparently unlabeled.

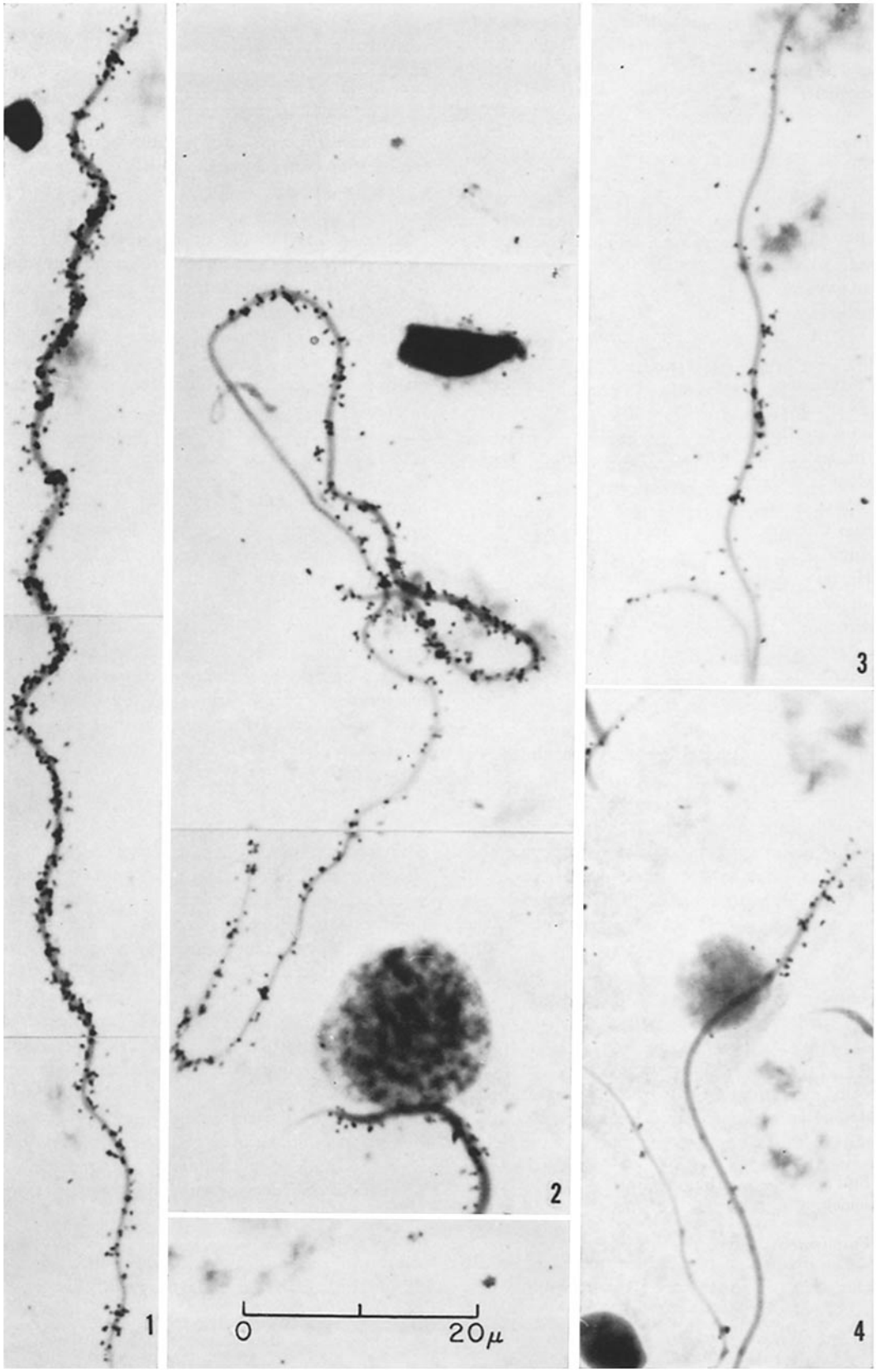
The cells with most of the H^3-thymidine incorporated into the DNA (deoxyribonucleic acid) of the X chromosome have been shown by Lima-de-Faria (1959) to be those which received the isotope in the late *S* phase (DNA synthetic-phase) of premeiotic interphase. In view of what is known of the labeling patterns in several other animals (Taylor, 1963), we may suppose that the X chromosome not only finishes DNA synthesis late, but begins synthesis late. Therefore, those cells without label in the X chromosome, but with most or all other chromosomes labeled, received H^3-thymidine in early *S*. Those with all chromosomes labeled—including the X, were at middle *S* at the time of injection of the isotope.

Examination of autoradiographs of mature or nearly mature sperms reveals patterns of labeling predicted on the basis of such out-of-phase replication of the X chromosome and a tandem arrangement of the chromosomes along the length of the sperm nucleus.

Maturing sperms are typically arranged in bundles or packets parallel to each other. However, squashing apparently breaks up some of the packets and scatters the sperms so that labeling patterns of individual sperm heads may be determined. Most of the sperms, of course, are unlabeled, but a few packets contain many labeled sperms. The patterns are of three general types as illustrated in Figs. 1 to 4. (1) Many of the sperms are labeled along the entire length (Fig. 1), especially in those animals which received two injections of H^3-thymidine separated by a 3- to 6-hour interval. (2) Another very common class for those receiving only one injection is shown in Fig. 2. An unlabeled gap appears somewhere along the length of the nucleus. Other unlabeled gaps may appear, but only one of them is long and clear. It probably represents the unlabeled X chromosome from cells receiving the isotope in early *S*. The unlabeled gap may be at any position. (3) The reverse pattern with only a single heavily labeled segment is seen in Figs. 3 and 4. Again, the labeled segment may occupy any position along the sperm nucleus. Sperms with this pattern are likely to be derived from spermatocytes in which most of the label was in the X chromosome, *i.e.* those at late *S* at the time of injection.

DISCUSSION

The results reported here indicate that the X chromosome and presumably all other chromosomes are arranged end to end in single file along the nucleus of the mature or nearly mature sperm. However, there is no visible Feulgen-negative gap separating the individual chromosomes. The chromosomes are very compact and cylindrical in cross-section. Since elongation of the nucleus is a slow process and the labeling patterns described above do not appear until the sperms are nearly mature, the chromosomes presumably slide past each other as the nucleus elongates. However, elongation continues in late stages of development after the tandem arrangement can be detected. The elongation is accompanied by a decrease in the nuclear diameter so that in the mature sperm the nucleus has a diameter of about ½ micron along the central portion. For comparison, the chromatids at anaphase I, fixed and stained by the same


1
2
3
4
0
20μ

procedure, have a diameter of about 1½ microns, and at metaphase II they are perhaps a micron in diameter.

On the basis of studies with polarized light, Inoué and Sato (1962) have presented evidence for a tandem arrangement of chromosomes in the sperm head of the cave cricket (*Ceuthophilus nigricans*). In addition, their evidence indicates that the chromosomes are arranged in a coil of a coil which is also the structure of the meiotic anaphase I chromatid of *Lilium* (Taylor, 1958) and presumably other species with large chromosomes.

Earlier studies by Hughes-Schrader (1946) demonstrated the tandem arrangement of the two chromosomes in the mature sperm of an iceryine coccid. Here, one chromosome regularly occupies a unique position, *i.e.* chromosome 1 migrates into the tail first, to be followed by chromosome 2. Cooper (1952) also reported observations of nearly mature sperm heads of *Drosophila* which indicate that the chromosomes are arranged end to end in single file along the long axis of the sperm. Herskowitz and Muller (1954) argued against such an arrangement, on the basis of the frequency distributions of lengths of sperm chromatin masses in two stocks of *Drosophila melanogaster*. They suggested that formation of sperm heads entail considerable overlapping of chromosomes. However, our autoradiographic results would rule out most, if not all, overlapping of ends in the mature grasshopper sperm. Although overlapping may occur during maturation, by the time the sperm head has reached two-thirds or three-quarters of its ultimate length the chromosomes appear to be sorted out in a tandem end-to-end arrangement.

This work was supported in part by Contract AT (30-1) 1304 with the Atomic Energy Commission at Columbia University, New York. The technical assistance of Miss Jeanne Tung is gratefully acknowledged.

Received for publication, January 10, 1964.

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Figure 1 Autoradiograph of a nearly mature grasshopper sperm stained by the Feulgen reaction and nearly uniformly labeled with H^3-thymidine.

Figure 2 Autoradiograph of a similar sperm in which an unlabeled gap (X chromosome) appears near the middle of the nucleus.

Figures 3 and 4 Autoradiographs of sperms in which the labeled region (X chromosome) appears some distance from the anterior end (Fig. 3) and at the anterior end (Fig. 4).