

THE SPERMATOGENESIS OF EUCHROMA GIGANTEA.

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The largest of the buprestid beetles, *Euchroma gigantea*, is native to Central and South America and is commonly found sunning itself on the trunks of trees. In such situations the beetles are not difficult to capture, as their movements are rather sluggish until they become thoroughly alarmed. The specimens from which the present study was made were taken at Culebra, Panama, in the month of August, at which time some of the beetles were mating, the male apparently attracting the female by a clicking sound produced by the elytra.

Upon sectioning the testes, I was surprised to find a complete series of stages from the spermatogonia to the mature spermatozoa, the younger stages not being confined to the larvæ or pupæ, as is frequently the case in insects. The testes were fixed in Gilson's mercurio-acetic-nitric solution or in Fleming's strong solution and stained with iron-hæmatoxylin or with saffranin and malachite green.

In the development of the germ cells of insects, as is well known through the results of the researches of Montgomery, Wilson, Stevens and others, there are present chromosomes which have been called heterochromosomes or idiochromosomes. Wilson (1909) has shown for the Hemiptera that in certain forms the idiochromosomes are equally well developed in both sexes, in others the male possesses one well developed, the other reduced in size, while in still others one is entirely lacking in the male. Stevens (1906) found somewhat similar conditions in the Coleoptera. Thus, the Elateridæ and Lampyridæ possess only the odd chromosomes, while the families Chrysomelidæ, Coccinelidæ, Scarabidæ, Silphidæ and Buprestidæ show one of the idiochromosomes reduced in size. In Carabidæ some members have an unequal pair of heterochromosomes, others an odd chromosome. *Euchroma gigantea*, as a member of the family Buprestidæ, belongs in the second of these groups (Figs. 21-22).

Besides the idiochromosomes, Wilson discovered in the Hemiptera a pair of chromosomes equal in size but noticeably smaller than the others, which he designated as *m*-chromosomes. According to the researches of Stevens these are occasionally present in the Coleoptera, *i. e.*, in *Trirhabda virgata* and *T. canadense* and in an unidentified buprestid. They likewise are represented in *Euchroma* (Figs. 19, 23, 24). In addition there are, in the spermatocytes, eleven chromosomes of more nearly equal size, making the total reduced number thirteen.

In most forms heretofore studied, the idiochromosomes are evident not only at the time of mitosis but also in the resting stage and prophases, for while the other chromosomes become resolved into the nuclear network, the idiochromosomes remain compact. It is in the manner of formation of the chromosomes during the prophases of the first maturation division and in the fact that neither at that time nor in the previous stages are the idiochromosomes distinctly different in behavior from the other chromosomes that the chief interest of the spermatogenesis of this beetle lies.

The nuclear network of the last generation of spermatogonia is of delicate texture. Chromatin masses occur at intervals, at first few in number and without constancy of position or shape (Fig. 5). The masses gradually become more distinct and form elongated threads near the center of the nucleus (Figs. 6, 7). The network breaks away from the nuclear wall and the synapsis is inaugurated (Figs. 8, 9). During this time there is no evidence of the idiochromosomes being isolated from the synaptic threads or failing to take part in their formation, nor, in the resting spermatocyte, do the idiochromosomes differ from the others. Stevens (1906) has reported a somewhat similar condition in the beetle *Tenebrio molitor*.

The nuclear network of the resting spermatocyte is more clearly defined than that of the spermatogonia and bears chromatin masses distributed with a fair degree of regularity (Fig. 10). This condition, however, does not continue. Instead of the usual spireme formation, the chromatin granules commence to migrate towards a specialized area within the nucleus (Figs. 11-14). The final result of this process is the formation of a

dense mass of chromatin in one part of the nucleus, the remainder of the nucleus being occupied by a fine network. It sometimes happens that more than one of these areas of aggregation develops (Figs. 15-18).

The outlines of the separate chromosomes may be seen, although they lie very close together. The nuclear network next begins to show a parallel arrangement of threads preparatory to the formation of the spindle (Figs. 17-18).

As is the case with other members of this order, the small heterochromosome is separated from the larger by the first division (Fig. 21). In mitosis the *m*-chromosomes tend to divide somewhat later than the others (Figs. 23-25).

After the second division the spindle fibers persist in the cytoplasm. They gradually cease to run parallel to each other, become more or less interlaced, and finally are arranged in spiral form (Figs. 26-28). Later they are converted into the tail of the spermatozoön (Fig. 32).

The chromatin of the spermatid at first condenses in large measure at the side of the nucleus nearest the spindle fibers, but as the latter lose their regularity of arrangement, the chromatin is dispersed throughout the network (Figs. 26-27). It next breaks into small fragments which migrate to the center of the nucleus, one mass, however, (probably the heterochromosome), remaining distinct from the rest (Figs. 29-32). As the nucleus approaches maturity, it elongates and the chromatin becomes finely granular, although fragments in chain form are still distinguishable (Fig. 33).

DISCUSSION OF RESULTS.

A number of workers (see Blackman, 1903) have described "chromatin nucleoli." They occur in a wide variety of plant and animal groups and usually during a period of growth. In oögenesis and spermatogenesis they may be present in the oö- and spermatogonia as well as in the later stages. Among the groups in which they have been discovered are echinoderms, molluscs, arthropods, amphibians and mammals, also in protozoa and plants. It may prove instructive to compare some of the more striking of these cases with that of *Euchroma*.

Sometimes the karyosphere is formed immediately after synapsis. Illustrations of this are furnished by the oögenesis of the dragonfly, *Plathemis* (McGill, 1906), and the pollen development of *Sarracenia* (Nichols, 1908). In this plant the karyosome is formed from the synaptic threads through an absorption of the chromatin substance by a nucleolus (Plate III., Fig. 1). The achromatic substance (linin) remains as a dense mass after the chromatin has passed by drops into the closely contiguous nucleolus. There is, therefore, a rather conspicuous separation here of chromatin and linin.¹

In *Plathemis* the synaptic threads gather closely around the nucleolus and form what Miss McGill calls a double nucleolus (oxyphil+basophil). There is plainly an interchange of material between the two parts of the nucleolus and, as the network again expands, masses of basophil substance are elaborated within the nucleolus and pass out on to the network.

In *Euchroma* the transfer of chromatic material to the karyosome is less direct, inasmuch as the synaptic threads are first extended into the network of the resting spermatocyte (Fig. 10; Plate III, Fig. 4, *a*). The latter possesses no large nucleolus such as is present in the plant nucleus. Nevertheless a center of activity arises, towards which the chromatin passes and simultaneously also the linin, at first in the form of streamers radiating from the karyosphere (Figs. 11-12). Later, as the chromosomes become distinct within the karyosphere, the linin is incorporated with them (Figs. 16-18).

Blackman (1903) interprets the karyosphere of the spermatocytic prophase of myriapods as a mass of fine, granular filaments closely gathered about the accessory chromosome. As the nucleus approaches mitosis, the threads emerge from the karyosphere, shorten and thicken to become the mitotic chromosomes (Plate III, Fig. 3, *a* and *b*). There is here no separation of chromatic from achromatic substance, only a strong attraction for both to a definite region of the nucleus. As compared with *Euchroma* this tendency manifests itself much earlier, for the karyosphere has already begun to resolve itself into definite chromosomes in

¹A closely similar behavior of chromatin has been described for *Spirogyra* by Berghs (1906).

Scolopendra at a period when in *Euchroma* it is condensing (Plate III., Figs. 3 and 4). In *Scolopendra* there is likewise a karyosphere present in the spermatogonia, whereas it is lacking in *Euchroma*.

A somewhat different condition is described by Eisen (1900) for *Batrachoseps*. A karyosphere (chromoplast) is present in this object. At first it lies free in a vacuole, but later appears to attract "leaders" which might be compared to the radiating rays of *Euchroma* (Figs. 11-12).

Through these leaders small particles of chromatin are projected into the karyosphere and again emerge from it. The convergence of the leaders towards the karyosphere apparently corresponds to a synapsis stage, and as they finally break apart each chromosomal thread receives a portion of the karyosphere which is gradually distributed through the length of the thread¹ (Plate III., Figs. 2, *b* and *c*).

The condition in echinoderm eggs treated with Mg salts (Wilson, 1901) and in normal mouse eggs (Sobotta, 1895) is in some degree similar to that of *Euchroma*, for although there is apparently no separation of linin and chromatin, the karyosphere breaks up directly into the mitotic chromosomes (Plate III., Figs. 5, *a* and *b*).

What bearing have these facts on the question of the individuality of the chromosomes? In the paper on *Sarracenia* I suggested that the phenomena there "might be explained on the assumption that the morphological basis of the chromosomes remains in the linin while that portion of their substance which causes them to color deeply is absorbed by the nucleolus. If a similar interpretation be applied to the case of *Batrachoseps*, it will be seen that the linin retains its individuality more clearly than the chromatin, which may be transferred to the karyosphere. In the myriapods there is apparently no separation of chromatin and linin, but a tight coil of threads, consisting of

¹Janssens (1905) in his later work on *Batrachoseps*, remarks: "il nous semblait très probable que les chromoplastes resultaient de la soudure très intime des chromosomes aux telophases à la faveur de rapprochement." "Il semble qu'une substance intensément siderophile, une sorte de nucleine, soit venue empâter tout le pole de la figure centre à ce moment. Il se peut qu'il s'agisse là d'un exsudat des chromosomes eux-mêmes."

both, forms the karyosphere. Possibly this might be regarded as a continuation and exaggeration of the synaptic condition, and, if this were true, a series of consecutive stages might be conceived between the typical synapsis and post-synapsis of most animals and plants and the extreme conditions presented by *Sarracenia*.

In the echinoderms, in *Mus* and in *Euchroma* a similar tendency to condense reappears and in the germ cells later than synapsis, owing, no doubt, to a chemical condition of the nucleus varying from the usual type. Here there is in one case (*Euchroma*) a more rapid condensation of chromatin than of linin and a consequent partial separation, while in the other (*Mus*, echinoderms) no such separation is apparent, chromatin and linin condensing simultaneously (Plate III., Figs. 4 and 5).

There is, however, little reason to believe that the difference in the method of formation of the spermatocytic chromosomes of *Euchroma* and most other insects is fundamental. The gradual change in coloration as resting chromatin becomes active goes to show that there is a chemical change in progress from less acid to more acid condition, accompanied by a condensation of substance. According to the differing constitution of different nuclei, this chemical activity might be confined to one center or distributed through the nucleus around several centers. If the latter were the case, the network of resting spermatocytes would break at various points and, condensing, form the chromosomes, but if there were but one center, the condensation would occur within a more circumscribed area. In *Euchroma*, while the latter method is more common, it may happen that the centers of condensation are multiplied (Fig. 15).

An interesting question concerning the relation of the chemistry of the nucleus to the individuality of the chromosomes presents itself at this point. If it be true that the aggregation of chromatin is accompanied by a decomposition of nucleo-proteids and a reduction of chromatin to nucleic acid or a simple compound of that acid and also true that the chromatin may be separated from the linin and gathered into a karyosphere, may it not be possible that the linin network is not homogeneous as regards its chemical character, but that in different areas are

developed different proteid substances which, when combined with the nucleic acid of the karyosphere, become active and colorable by chromatin stains. It may be that usually in the development of the germ cells the nucleic acid becomes chemically dissociated, but not visibly separated from these proteid substances, and consequently no karyosphere is present, simply chromosomes consisting of a condensed linin framework surrounded by nucleic acid.

PHILADELPHIA NORMAL SCHOOL,
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EXPLANATION OF PLATE I.

The drawings were made with the camera lucida, Zeiss microscope, oc. 12, obj. oil immersion 1/12. They have been enlarged to twice the diameter and reduced one half.

FIGS. 1-4. Spermatogonia. Prophases.

FIGS. 5-7. Last generation of spermatogonia. Prophases.

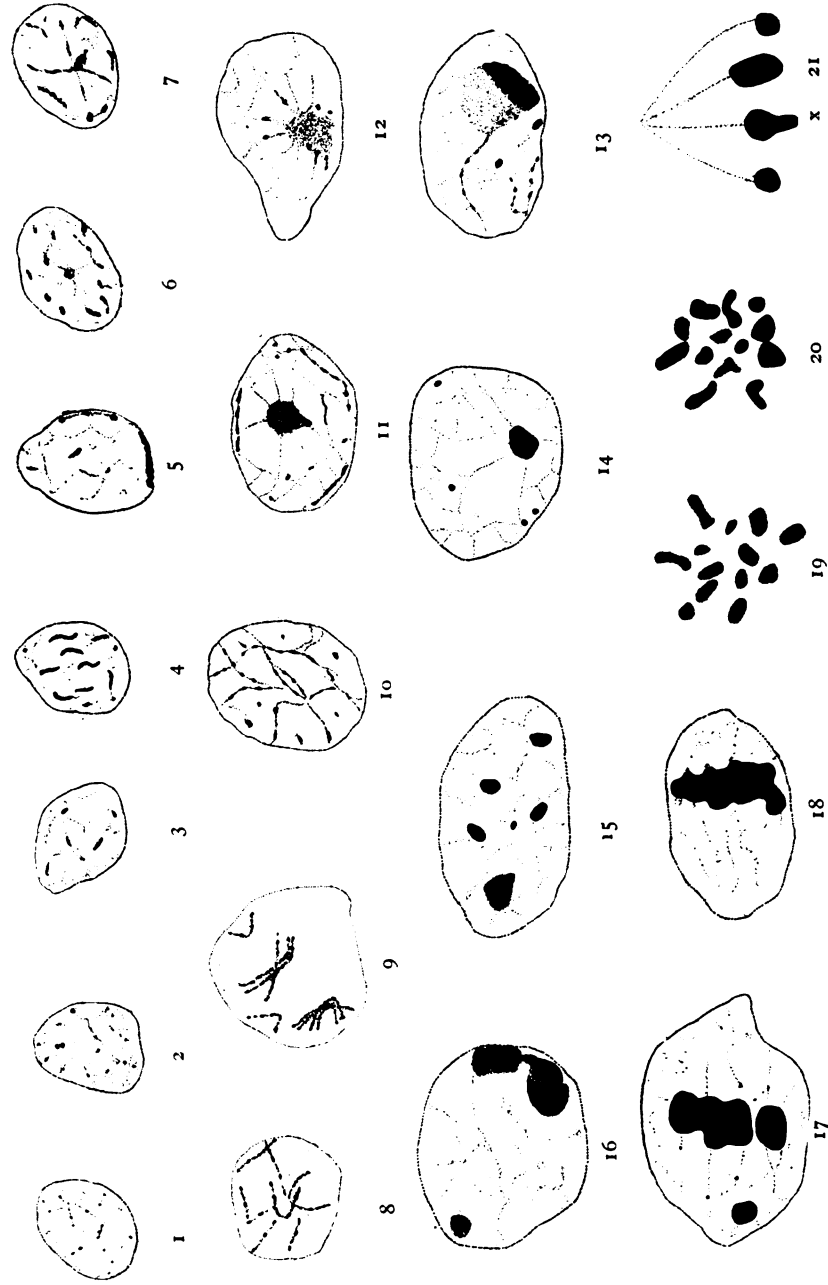
FIGS. 8-9. Synapsis.

FIG. 10. Resting spermatocyte.

FIGS. 11-18. Spermatocytes. Prophases of the first maturation division.

FIGS. 19-20. Equatorial plates of the first maturation division. 13 chromosomes.

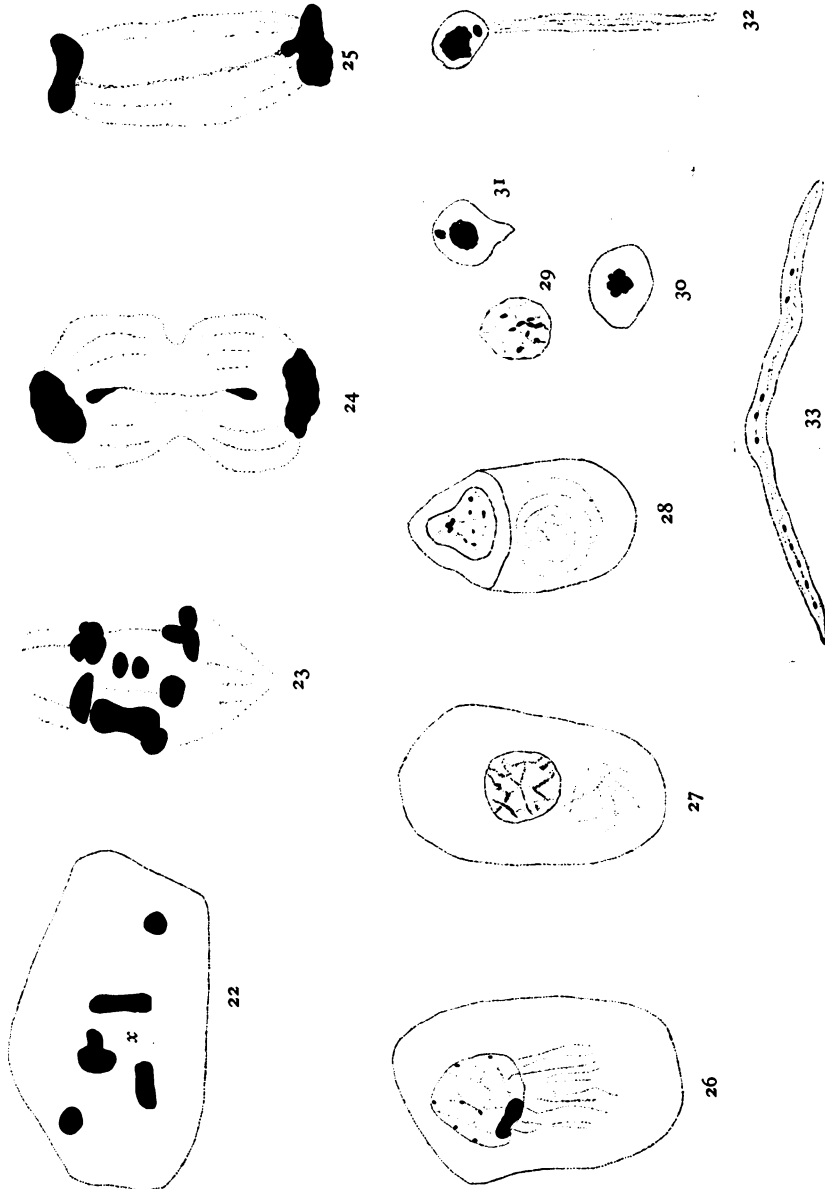
FIGS. 21. The heterochromosome x.



M. LOUISE NICHOLS.

EXPLANATION OF PLATE II.

- FIG. 22. The heterochromosome x.
FIG. 23. Side view of the first maturation division. Metaphase.
FIG. 24. Anaphase. Late division of the microchromosomes.
FIG. 25. Telophase. Traces of the late division of the microchromosomes.
FIGS. 26-33. Spermatids.

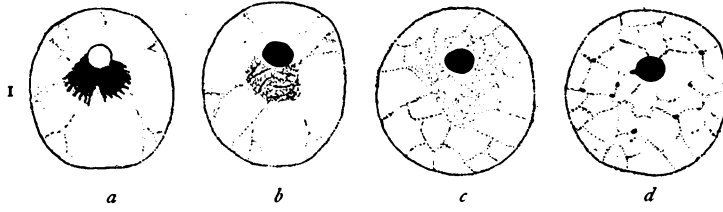


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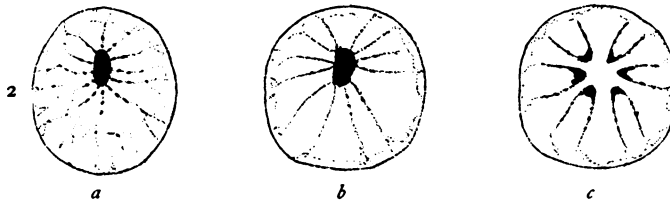
EXPLANATION OF PLATE III.

FIGS. 1-5. Diagrams showing the relation of the karyosphere to chromatin and linin.

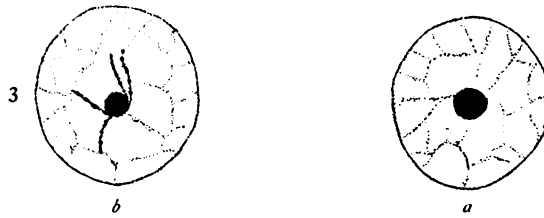
Sarracenia.



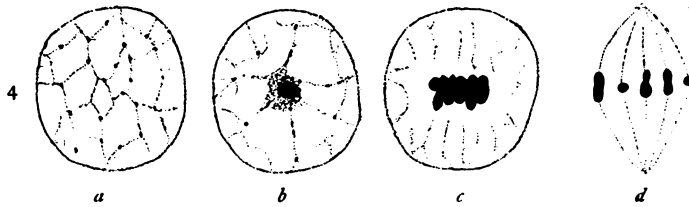
Batrachoseps.



Scolopendra.



Euchroma.



Mus. Echinoderm.

