A STUDY OF THE EARLY MATURATION STAGES

IN THE MALE AND FEMALE HOMOPHRON AMERICANUM

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A STUDY OF THE EARLY MATURATION STAGES IN THE MALE AND FEMALE HOMOPHRON AMERICANUM

THESIS

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CHAPTER I

INTRODUCTION

The Problem

Many investigators have made studies of the chromosomes and their behavior during spermatogenesis in the species of different representative families of the order Coleoptera. The emphasis was placed on the sex chromosomes (earlier called the hetero-chromosomes), chromosomal number, and size and shape of the chromosomes for the various species. No reference was found in the literature to cytological work on the sand beetle, Homophron americanum. This insect belongs to the family Homophronidae which has only the one genus, Homophron.

This small beetle is very prevalent on the sandy beaches of creeks and fresh water ponds in the region of North Texas and is found burrowing in small tunnels. These tunnels are located in the moist sand at a depth of approximately one inch. Therefore, abundant material can be collected for cytological studies.

This investigation was conducted in an attempt to determine first, the chromosomal number and types of sex chromosomes, and second, the maturation stages of the male and female.

Review of Literature

In reviewing the research that has been done concerning the behavior of the chromosomes in various species of the Coleoptera, it has been found that several investigators have placed special emphasis upon the study of the sex chromosomes or as they were called in earlier papers, hetero-chromosomes. These earlier studies also included the chromosomal count for the species, and a tracing of the partial or complete formation of the gametes.

Among the first papers referring to the chromosomal studies of the beetle was that of Stevens (1905) in which she demonstrated the chromosomal count and type of hetero-chromosome for thirty species of Coleoptera and found that the greater percentage of the beetles described adhered to the X - Y type of hetero-chromosome, and the others to the X - O type for the male.[1] This same worker (1906) continuing with the description of the hetero-chromosomes of fifty species of the families Coccinellidae, Chrysomelidae, Melandryidae, Meloidae, Cerambycidae, Staphylinidae, Silphidae, Elateridae, and Lampyridae as demonstrated from the polar spermatogonia, secondary spermatocytes, and metaphase stages found in agreement to her previous results that only twelve of this number possess the unpaired hetero-chromosome--, all of the

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[1]N. M. Stevens, "Studies in Spermatogenesis With Especial Reference to the 'Accessory Chromosome'," Carnegie Publication, No. 6 (1905).

others having an unequal pair.[2] Stevens found that the spermatocyte stages were first found in the insects as the larve begin to pupate, and the somatic mitosis was found to a greater advantage in the larval stages. The pupa too, contained the spermatogonial and early prophase stages of maturation.

While studying cytologically these fifty species, two distinct types of synizesis and synapsis were found in the genus Photinus (Family, Lampyridae). First, in the loop type, synizesis seems to be a prolonged telophase of the first spermatogonial mitosis, the spermatogonial number of chromosomes appearing as short loops crowded together at one end of the nucleus. After a time, the loops straighten and the free ends unite in pairs and the pairs unite to form a spireme. In some cases the synapsis stage is very distinct; in others, synapsis and union to form a spireme occur nearly or quite simultaneously.

The second, or spireme type of synizesis, is preceded by synapsis which may form a distinct stage as in Photinus pennsylvanicus and Lineus grisens, or it may occur in the anaphase or telophase of the last spermatogonial mitosis, and a closely wound spireme follows immediately. In this latter type, the hetero-chromosomes are usually distinguishable in the synizesis stage outside of the massed spireme, while

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[2] N. M. Stevens, "Studies in Spermatogenesis; Sex Determination," Carnegie Publication, No. 6, Part II (1906).

in the first type, they are not seen until after the spireme has been formed.

Stevens (1909) further reports that she found the hetero-chromosomes of three species of Diabrodica to have the unpaired hetero-chromosomes in the male, presumably of the X - 0 type, which divides in the second maturation division but not the first. Synapsis occurs at the close of the synizesis stage. Also the interesting phenomenon of the so-called "super-numerary" chromosomes of uniform size were described in the germ cells of 50% of the group of Diabrodica soror and 12-punctata which divided at the maturation stages. However, there was no indication of an association of this fact with any variation in external characteristics of the insects.[3]

In agreement with Stevens' work on the hetero-chromosomes, Goldsmith (1919) found that a greater number of the species of the family Cicindelidae which he examined to possess the X - Y type rather than the X - 0 type in the male germ cells. During his study of the chromosomal behavior, and a statement of the chromosomal numbers and shapes for each species, he failed to discuss in detail the important prophase stages of spermatogenesis.[4]

Consideration of another phase of the spermatogenesis

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[3] N. M. Stevens, "Chromosomes in Diabrodica," Jr. Exp. Zool., II (1909), 275-303.

[4] W. M. Goldsmith, "A Comperative Study of the Chromosomes of the Tiger Beetles (Cicandelidae)," Jr. Of Morph., LVX(1919), pp. 341-362.

of the Coleoptera was undertaken by Bowen (1924) in which was presented the description of the spermatid and spermatozoa formation of three species of Coleoptera: Chelymorpha cassidea, Lixus concavus, and Cicindela segatta. The latter species was studied from the original slides of Goldsmith. The general type of sperm described for these insects is the flagellate.[5] All three species correspond in general to the rule of flagellate spermatozoa formation; i. e. formation of nebenkern, centrioles, golgi bodies, concentration of the chromatin, and eventual elongation of the sperm and location of the acrosome — each having the same fate as described for the development of the spermatid and spermatozoa in the Hemiptera by Bowen (1923).[6]

Hayden (1925) contributed to the cytology of the Coleoptera a study of the growth phases in the male of the beetle Phanaeus, in which the twelve V-shaped leptotene threads are polarized with their distal ends in another body (primary and secondary caps, respectively), to undergo a conjugation of the parasynaptic type. The distal ends of the chromosomes are freed from their attachment in the secondary cap. The primary cap apparently becomes incorporated again in the chromosomes; the secondary cap, together with linin remnants

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[5] R. H. Bowen, "Studies on Insect Spermatogenesis. VI Notes on the Formation of the Sperm in Coleoptera and Aptera, with a general discussion of Flagellate Sperm," Jr. of Morph., II (1924), 351-372.

[6] R. H. Bowen, "Studies on Insect Spermatogenesis. I Components of Spermatid and Their Role in Formation of Sperm in Hemiptera," Jr. of Morph. XXXV (1923), 179-208.

of the pachytene chromosomes, disintegrate in the nucleus as residual chromatin.

In the dissolution of the karyosphere six ring shaped tetrads emerge arranged in a temporarily connected chain, giving under certain conditions, the misleading impression of twelve components arranged end to end. The entire content of the karyosphere appears to be employed in the formation of the chromosomes; no visible plasmosome remains.[7]

The first complete general account of the spermatogenesis of Coleoptera is given by Brauer (1928) of Brushus quadrimaculatus Fabr. Briefly, the spermatogonia undergo two mitotic divisions. After the second division, the nuclei remain small and very dense for some time before the beginning of the growth phase. During this interval, the nuclei do not assume again the characteristics of the interkinesis stages.

In the primary spermatocytes typical tetrads are formed. The chromosomes are symetrically V-shaped. The end of one arm of the "V" fuses with the end of the corresponding arm of its synaptic mate. This fact compares with the similar synapsis formation of the chromosome chain in Phaneus as described by Hayden. In agreement with Bowen's explanation for Chalymorpha, Lixus, and Cicindella, Brauer finds that

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[7]Margaret Hayden, "Karyosphere Formation and Synapsis in the Beetle, Phaneus," Jr. of Morph., XL (1925), 261.

after disjunction takes place in the primary spermatocyte division in Bruchus and after the division of the secondary spermatocytes has taken place, the chromosomes become vesicular and form a retieular nucleus in the spermatid. Immediately following the chromatin becomes deposited as a chromatin ring around the nuclear periphery.

Like Stevens (1909), Brauer finds that an unpaired X-chromosome is present in the spermatogonia and in male somatic cells, which fails to divide in the primary spermatocyte division, but passes as a whole to one pole in advance of the autosomes, indicating the X -0 type of sex chromosome. The X xhromosome divides normally in the secondary spermatocyte division with the autosomes.[8]

Not until Varde (1930) studied the morphology and cytology of the ovaries of Dytiscules pisanus and several related species in regard to the development of the eggs was there any work done on oogenesis of the beetle. Stevens and Goldsmith used the significant polar views of the primary oocytes only to demonstrate the type of sex chromosomes that the species possessed. Varde found that the ovaries were differentiated into five zones: (1) multiplication,

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[8]Alfred Brauer, "Spermatogenesis of Brachys Quadrimaculatus," Jr. of Morph., XLVI (1929), 217-231.

[9]V. P. Varde, "Contribution a l'etude de l'ovogenesis des insectes; l'ovogenesis des Coleopteres dytiscules," Archeais Biologies, XXXI (1930), 5-112.

(2) pre-differentiation, (3) differentiation, (4) little growth (subzones synaptic and growth), (5) ovarian tube (subzones of great growth and beginning of maturation). The cytology of these zones was also described. An examination of the earlier maturation prophases revealed that synapsis begins immediately after the fourth pre-oocyte mitosis. Also the germinative vesicles of the oocytes do not in a great measure contain chromatin.

CHAPTER II

MATERIALS, METHODS, AND OBSERVATIONS

Materials and Methods

The materials used in this investigation consisted of male and female gonadal tissue of the sand beetle, Homphron americanum, specimens of which were collected weekly from the sandy banks and beeches of the drains and streams flowing into Aubrey pond (located approximately nine miles northeast of Denton, Texas) from June 13 until August 11, 1938.

Collections were made by two methods: either by pouring water on the already moist banks, allowing it to infiltrate approximately an inch below the surface in order to flood and collapse the small tunnels, and thus force the beetles to come to the surface; or, by tramping the sand and forcing the beetles to come to the surface by flooding the burrows with capillary water.[1] These were caught, carried to the laboratory, head removed (thus withdrawing the digestive tract also), and preserved entire after splitting the chitinous covering of the back in order better to allow the fixing agent to penetrate the tissue.

Bouin's and Flemming's fixing solutions were used for

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[1] J. K. Gwynn Silvey, "An Investigation of the Burrowing Inner-Beach Insects of Some Freshwater Lakes," Papers of Mich. Acad. of Science, XXI (1936), 656.

preservation; however, best fixation was obtained by placing the tissue in Bouin's with 2% urea crystals added and in Allen's modification of Bouin's B-15, from four to six hours. Varying grades of alcohol and xylol were used for dehydration, hardening, and clearing respectively. More complete hardening was secured by leaving the tissue in 80% or even higher grades of alcohols for as much as twenty-four to forty-eight hours. Dissection of the gonads and reproductive tract was also made while the tissue was in 80% alcohol.

After imbedding in paraffin, the material was sectioned at 4 micra and stained in Heidenhain's iron-hematoxylin. Use of eosin as a counter stain proved ineffective.

Observations

Description of the Reproductive Organs

Female.--The gonads of the female of Homphron consist of six ovarioles in the shape of slender filaments suspended by a suspensory filament which appears to be imbedded in the neighboring fat tissue as no connection to the diaphragm or body wall was observed. The ovarioles are held enclosed together in a peritoneal sheath of connective tissue. The principal divisions of each ovariole consist of the terminal filament followed posteriorly by the egg tube which leads into the lateral oviduct by means of the stalk-like pedicel. A union of the lateral oviducts from each ovary form the median oviduct or oviductus terminalis that in turn empties into the genital chamber. The spermotheca also has its

opening into the genital chamber.

In the subdivisions of the egg tube are found the more significant early maturation stages. These are the germarium (containing the germ cells and young oocytes as well as oogonia and nurse cells), and the egg chambers, which are formed by further distension of the vitellarium into follicles that become large toward the posterior end of the tube. The follicular walls are composed of small follicle cells, a few of which were found in addition to the oogonia and nurse cells in the germarium. By the time the egg chambers are reached the nurse cells have disappeared. This characteristic places the ovarioles definitely as the panoistic type of ovary. No well-defined trophocytes are found.[2]

Male.—Each of the paired testes, resembling small sac-like organs suspended in a peritoneal sheath continuing from the fat tissue near the diaphragm, consists of several sperm tubes which contain the male germ cells in successive stages of development and other cells associated with the germ cells in various capacities. Each tubule is attached to the vas deferentia of each testis and is considerably coiled ending in a union to the ductus ejaculatoris that in turn extends into the copulatory organ.

The wall of a testicular tubule consists of a cellular

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[2]Terminology for the description of the gonads taken from R. E. Snodgrass, Principles of Insect Morphology, p. 550.

epithelial sheath of only one layer in thickness. Within the tube are consecutive compartments or cysts of developing germ cells, each separated by an eipthelial fold.[3]

Early Stages of Oogensis

Located nearby and within the terminal filament are the oogonia. The oogonia (Fig. 1) undergo several multiplication divisions (apparently two) before beginning the growth into the primary oocytes (Fig. 4). The density of the chromatin granules and the close clumping of the chromatin threads as well as that of the chromosomes make it difficult to observe in correct detail the mitotic divisions of the oogonia; however, the spindle figures (Fig. 2 and 3) demonstrate the general behavior of the nuclear material within the cell. No centrosome is present in these stages.

Chromatin within the resting primary oocyte (Fig. 4) begins to form a typical leptotene figure consisting first of a thin, lightly staining spireme which eventually shortens and thickens into a more dense and plainly visible spireme coiled throughout the nucleus (Fig. 5). At this point the nucleus has become larger and has lost all trace of a nuclear membrane, the entire cell being filled with the early maturation activity. The developing oocytes were much clearer in detail than the oogonia due to the continual growth of the cell as the propheses of maturation progressed. The two

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[3] Ibid.

small groups of chromatin that are identifiable in the resting oogonia and also the resting oocytes (Fig. 1 and 4) seem to lose their identity as such and enter into the spireme along with the diminuted chromatin.

The spireme, concentrating and thickening further, separates into two groups of paired threads, a zygotene stage (Fig. 6). Gradually, these two groups move in closer to each other and become more intertangled to become pachytene in nature (Fig. 7). Immediately following within the next follicle group, the chromosomes are found emerging from this syndesis and can be recognized as paired homologues distributed throughout the nucleus (Fig. 8). Within the bouquet stage just described a closer observation reveals that a portion of the chromatin spiremes has broken into threadlike fragments, which become more identical as the paired homologues (Fig 8). This formation of the homologues is so progressive that no true diplotene could be distinguished at this time. The chromosomes become more lengthened and the ends all fuse in a cap-like plasmosome giving the appearance of a number of loops (Fig 9).

Again, staining was lost and a confused staged ensued as a period of great growth set in. A definite boundary or nuclear membrane was laid down and the chromosomes, which last appeared as the loops of synizesis, reappeared after the confused stage as vacuolated chromatin threads, which retained the well-defined shape and number that they were in Fig. 8.

Yet, the homologues appeared separately in two groups as may be observed by focusing up and down in order to view the entire depth of the cell. (Fig. 10).

The egg now approached the germinal vesicle formation. The vacuolated chromatin threads appeared to concentrate together in a large heavily staining nucleolus. Nevertheless, some of the chromatin remained residual in the nucleus. Throughout the continued growth of the oocyte this nucleolus was located towards one side of the oval shaped nucleus. At the beginning of this great growth, only three to five cells were within a follicle; and when the development was climaxed as the germinal vesicle, only one large cell was found within a follicle. The germinal vesicle (Fig. 11) acquired a covering consisting of the impression of or probably made up of the follicular cells.

Slides made from the ovaries of the follicular cells in June showed the beginning growth stages but not the period of great growth just described. This first appeared in the group collected about three weeks later, July 2, 1938. The last collection, August 11, 1938, showed many more follicles which contained growing oocytes that were still in the germinal vesicle stages.

Later Stages of Spermatogenesis

The testes dissected from the males collected June 13 were too old to secure dividing spermatogonia and the important prophase I stages of maturation. However, they did

contain good diakinesis stages of the primary spermatocytes and the successive maturation stages to the formation of the spermatids and their metamorphosis. After July 21, nothing but secondary spermatocytes, spermatids, and an enormous increase in the number of mature spermatozoa were found.

The diakinesis of the male consisted of a series of tetrad-appearing figures within the nucleus; however, closer examination demonstrated a group of single, compact chromosomes which were in very close contact with each other, only two of the tetrads appearing as heterotypic chromosomes (Fig. 13). These tetrads line up in the center of the cell preparatory to reduction division or separation of the homologous chromosomes (Fig. 14). After the reduction division the chromosomes appeared singly in the polar secondary spermatocyte stages (Fig. 16 and 17), but soon underwent a mitotic division (Fig. 15) to form the spermatids.

Spermiogenesis

The spermatid assumed a spherical form immediately following the telophase stage at the close of the mitotic division of the secondary spermatocytes. The typical resting spermatid consisted of a small spherical cell with a darker stained nucleolus and an aggregation of the chromatin near the periphery of the cell. As metamorphosis began the cell soon grew more filiform in shape. The acrosome of the mature spermatozoa was formed from a portion of the spindle of the last telophase division that rotated around the nucleus until it reached the

anterior end as shown in the drawing (Fig. 18). The acrosome and nuclear content formed a head piece with a long barb-like hook extending posteriorly that in the more mature spermatozoa stained very darkly. In the early spermatid, no centriole was visible at first; however, near what will eventually be the posterior or lower end of the nucleus, one appears with a small mass of protoplasm. This small mass of protoplasm which appears to have its origin from the material within the cell of the secondary spermatocyte before the mitotic division forms the spermatid, is observed to form the sheath surrounding the tail or flagellum of the mature spermatozoon. The centriole is either two when it appears in the spermatid or it divides later to form two that can be observed in the lightly stained mature spermatozoon. From the distal centriole the flagellum was formed and enclosed inside the sheath with which it fuses so closely that no evidence of the sheath could be observed later.

No middle piece was present, the nucleus and centrioles forming the main body of the spermatozoon with the long flagellum attached posteriorly.

Chromosomal Number and Sex Chromosomes

The chromosomal number was determined in the female from the growth stage in the ovary (Fig. 8) in which seven pairs of homologues were present giving a total diploid count of fourteen. By a careful examination these may be observed again in the vacuolated chromosome stage as portrayed in the

formation of the germinal vesicle (Fig. 10). Still more evidence that the diploid count is fourteen may be secured from a count of the chromosomes of the polar oogonium (Fig. 12).

In the male this count was determined from an examination of the diakinesis stage in which ten single chromosomes were found and two tetrads consisting of two chromosomes each making the total number fourteen for the diploid count (Fig. 13). More conclusive evidence is found in the polar secondary spermatocytes each of which contained the haploid count of seven chromosomes completely separated from one another (Fig. 16 and 17).

There was found in the male germ cells two chromosomes which are not of the same size and shape. These never underwent synapsis as did the other chromosomes in the diakinesis (Fig. 13), thus presenting a good opportunity to study them. The first is a short, rather sharply curved chromosome, whereas the second is merely a small round body of chromatin.

It was assumed that these chromosomes represented the sex chromosomes for the following reasons: First, one of these chromosomes passes into one daughter secondary spermatocyte and the other into the second daughter cell as can be observed in the polar view of the secondary spermatocytes (Fig. 16 and 17). Second, one of the pairs of chromosomes consists of two homologues corresponding identically to the size and shape of the first short, curved unpaired chromosome of the female (Fig. 8). This assumption was borne out further

from an examination of the vacuolated chromosome stage in the development of the germinal vesicle, for the two definite, short, curved chromosomes are also present. According to this evidence the Homophron is of the X - X, X - Y, type of sex chromosome. The curved chromosome is interpreted as the X and the round chromosome as the Y.

Further study of the behavior of these sex chromosomes showed that they always, during all divisions of the cell preceded the autosomes in their migration from the equatorial plate to the poles, as is represented in the anaphase of the dividing oogonium (Fig. 2), that had been sectioned diagonally. This phenomenon was also shown in the metaphase of the oogonium (Fig. 3) and in the metaphase division of the secondary spermatocytes in their multiplication to form spermatids (Fig. 15). It was observed that the sex chromosomes of the spermatid were the last of the chromosomes to lose their identity in the formation of the chromatin granules preparatory to a concentration of the nucleus. The sex chromosomes during oogenesis lost their identity first in the leptotene stage of the prophases and regained it in the vacuolated chromosome stage preparatory to the formation of the germinal vesicle only to lose it when the germinal vesicle was reached.

CHAPTER III

DISCUSSION, SUMMARY, AND CONCLUSIONS

Discussion

In Homophron the two consecutive mitotic divisions of the oogonia substantiates the evidence reported by Varde (1930) that the oogonia undergo a definite number of preovocyte divisions before entering the resting stage preparatory to growth into the primary oocyte (Fig. 1).[1] This definite number of divisions is reported by Stevens (1906), Goldsmith (1919) and Brauer (1929) in the spermatogonia of the species that they investigated.

The resting oogonia (Fig. 1) of Homophron began immediate growth into the resting oocyte without any pause or rest period. This immediate change was found also in Dytiscus, Varde (1930). On the contrary, Stevens (1906) and Hayden (1925) found in the species which they examined a long rest period of the spermatogonia before growing into the resting spermatocytes.

Study of the growth from the resting oogonia (Fig. 4) into the primary oocyte of the sand beetle reveals that there were two periods of growth. A period of little growth occurred in which the chromatin underwent the principal pro-

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[1] V. P. Varde, Op. Cit., pp. 5-112.

phases of maturation from the formation of the resting oocyte (Fig. 4) to the paired homologue stage (Fig. 8). This period of little growth involves only the growth of the nucleus. A period of great growth follows which continues from the confused stage entered into by the paired homologues to the germinal vesicle (Fig. 11). Cytoplasm becomes visible and by the time the germinal vesicle stage is reached a covering of follicular cells is beginning to surround the cytoplasm. This description is in accord with the results of Verde (1930) who stated that there are two growth periods in Dystiscus, a period of little growth and a period of great growth.

However, Stevens (1906) and Brauer (1929) found that in spermatogenesis, the formation of the primary spermatocytes from the resting spermatocytes consisted of only one continuous growth. Probably, this difference in growth is accounted for by the great deposition of yolk that is necessary in the formation of the egg of most insects. Bowen (1923) found this to be true in certain species of the Hemiptera.

Chromatin of the resting oocyte (Fig. 11) of Homophron forms the spireme which develops into a pachytene or synapsis stage (Fig. 7) from which paired homologues are formed (Fig. 8). Synizesis follows afterwhich the chromosomes lose their staining capacity and appear in a confused stage. These prophase changes are not in agreement with t e description of the chromatin behavior of the prophases of Dystiscus as described by Verde (1930), for in Dystiscus the synizesis follows the

synapsis. Hayden (1925) described a synapsis following the spireme formation, and an immediate changing into the synizesis during the spermatogenesis of Phanaeus. The wide variation in these results is not unusual, however, for Wilson (1925) found in general insect spermatogenesis that the synizesis may occur at any stage of development from synapsis to the close of the diplotene stage, varying with the species.[2] As can be seen from a discussion of the literature, this variation in the time of occurrence of the synizesis occurs even among members of the same order.

The synizesis of Homophron is of the same loop type as that described by Stevens (1906). The ends of the chromosomes fuse into a plasmosome-like body (Fig. 9). In the spermatogenesis of Phanaeus, Hayden (1925) found this type of synizesis also.

Previous to the period of great growth, chromosomes in confused stages were found in Homophron and showed no results of staining, which after a laying down of the cytoplasm resulted in the vacuolated chromosome stage (Fig. 10). In this vacuolated chromosome stage, the chromosomes are spread in two groups throughout the nucleus. These chromosomes move closer together and concentrate into the darkly staining nucleolus of the germinal vesicle stage (Fig. 11). No description of a

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[2]Edmund B. Wilson, The Cell in Development and Heredity (1925), p. 368.

confused stage is found in the results of Verde (1930), even though Homophron oogenesis otherwise closely corrobates his description of the formation of the germinal vesicle. This vacuolated chromosome stage (Fig. 10) was interpreted by Stevens (1906) as a diplotene stage because of an identical resemblance to the diplotene formation described in the male species with which she worked.

At the time that collections of Homophron were first made, spermatogenesis had progressed to the diakinesis stage (Fig. 13) with the chromosomes in tetrads. These tetrads line up on the spindle and separate into the respective secondary spermatocytes (Fig. 16 and 17). Without any intervening stage, the chromosomes line up on the spindle again and undergo a mitotic division or longitudinal splitting to form spermatids (Fig. 15). The typical spermatid consists of a spherical cell with a nucleolus in the center and a distribution of the chromatin near the periphery of the cell (Fig. 18). This description is in accord with that given for the spermatid of Termopsis by Stevens (1905)[3].

The primary spermatocytes showing diakinesis were very large, but after the reduction division of the chromosomes the secondary spermatocytes were reduced one-half the size of the primary spermatocytes. The spermatids continued to shrink without any apparent sloughing off of cytoplasm that usually accompanies the metamorphosis of the spermatid. Stevens (1905) reported like behavior in spermiogenesis of Termopsis.

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[3] N. M. Stevens, Op. Cit. (1925)

Significant stages of the spermiogenesis of Hemophron indicate that the spermatids gradually became more filiform in shape. At one side of the spermatid two centrioles appeared to rise in situ (Fig. 15). From the distal centriole a simple flagellum was formed. The spindle fibers of the telophase of the second division gradually migrated to the anterior end of the spermatid to assume the appearance of a barb-like acrosome that fuses with the nucleus to form one large head piece consisting of the acrosome, nucleus and centrioles. No middle piece was observed in the mature spermatozoan. The sheath that surrounds and fuses with the flagellum originated from the small amount of cytoplasm that surrounded the spermatid. This description agrees in general with the metamorphosis of the flagellum sperm as described by Bowen (1925).

There were observed in the polar oogonia fourteen small compact chromosomes and in the diakinesis of the male fourteen chromosomes. Yet, the most accurate chromosomal count may be obtained from the paired homologues of the developing primary oocyte (Fig. 9). In this stage, the proportional sizes and shapes were also clearly identified. A still further presentation of the diploid count is visible in the vacuolated chromosomes or diplotene stage of the developing germinal vesicle. Stevens (1909) used in addition to the stages mentioned above the polar spermatogonial stages and polar views of the primary spermatocyte to determine the diploid count of the chromosomes for Diabrotica.

The haploid count was found to be seven in Homophron from the polar secondary spermatocytes wherein the chromosomes were evenly distributed throughout the cell.

Homophron belongs to the group of Coleoptera that has been described cytologically to be of the X ~ X, X ~ Y type of sex chromosomes. Each secondary spermatocyte contained an odd chromosome(Fig. 16 and 17). In one cell was seen spherical chromosome, and in the other a sharply curved chromosome was observed. Two of these sharply curved chromosomes were visible in the growth stage that followed the late pachytene. These were also visible in the later growth stage where the chromosomes were vacuolated. In diakinesis when the diplotene appeared in syndesis, the sex chromosomes were visible as seperate chromosomes.

Summarizing this information, it can be assumed that the sharply curved chromosome is the X since it is paired in the germ cells of the female Homophron, and the spherical body of chromatin is the Y due to the fact that it is only present in the male germ cells.

In Homophron, the sex chromosomes preceded the autosome on the mitotic spindle. Too, the unlike sex chromosomes fail to come together or near each other in the diakinesis of spermatogenesis, and during the resting stages of oogonia, oocytes, and the spermatid, they are visible either singly or in pairs while the other chromosomes have apparently broken up into chromatin granules. These results substantiate the

description of the behavior of the sex chromosomes as found in other Coleoptera by Stevens (1909), Goldsmith (1919), and Brauer (1926).

Summary and Conclusions

1. Early oogenesis of Homophron americanum is traced from the resting oogonia and their divisions to the formation of the germinal vesicle stage.

2. In spermatogenesis of Homophron, the primary spermatocytes are found to undergo two divisions to form the spermatid. The first of these divisions is the reduction division, and the second is a longitudinal division.

3. The representative stages of the metamorphosis of the spermatid into the mature spermatozoa are studied.

4. The diploid chromosome count for Homophron is determined and the diploid chromosome count is found to be fourteen. The type of sex chromosome for the female is of the X - X type and that for the male is the X - Y type.

PLATE I

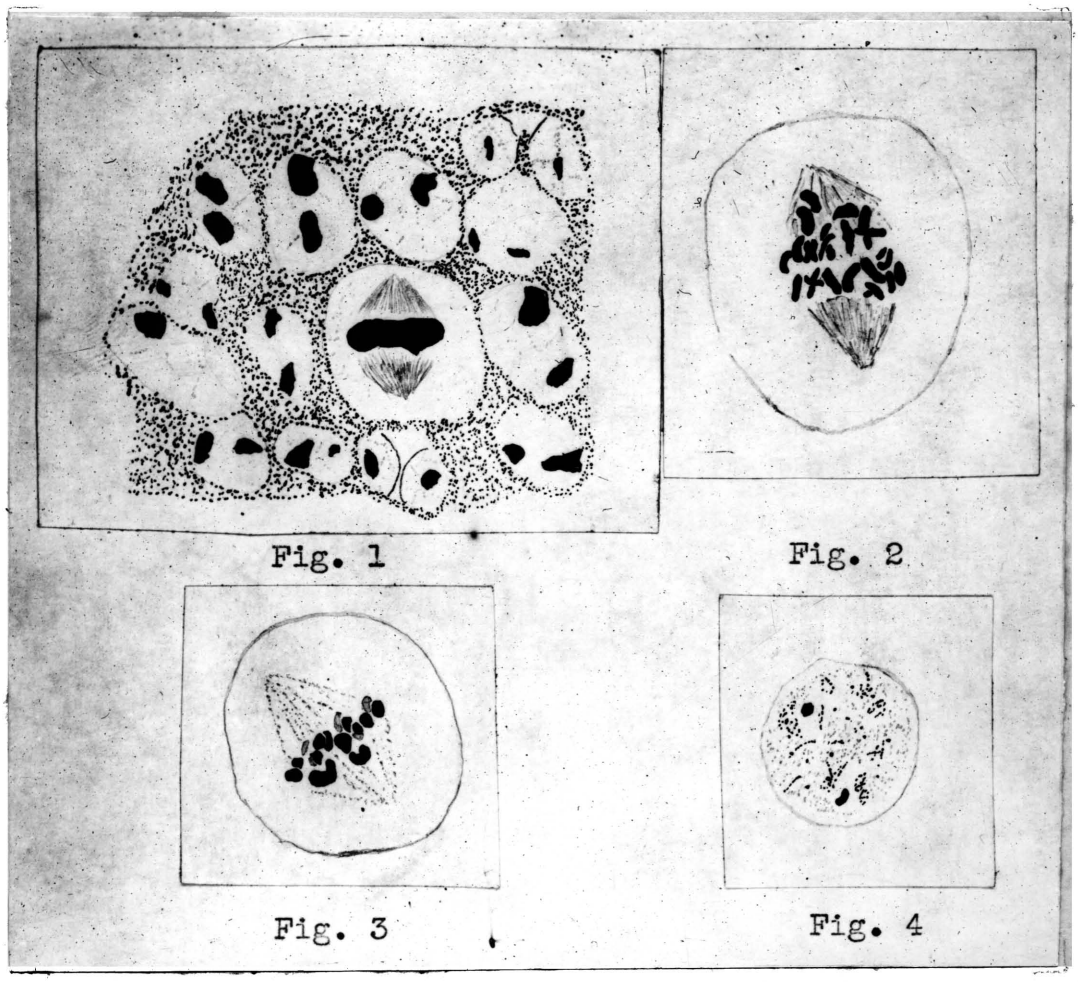

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Camera lucida drawing of a group of oogonia showing one oogonium with the chromosomes lined up in the equatorial plate on the spindle.

Fig. 2. Camera lucida drawing of late metaphase of an oogonium showing the sex chromosomes in advance.

Fig. 3. Cmaera lucida drawing of an oogonium sectioned obliquely in which the chromosomes are lined up on the spindle. The sex chromosomes are in advance.

Fig. 4. Camera lucida drawing of a resting oocyte. Sex chromosomes are not diminuted into chromatin.

PLATE II

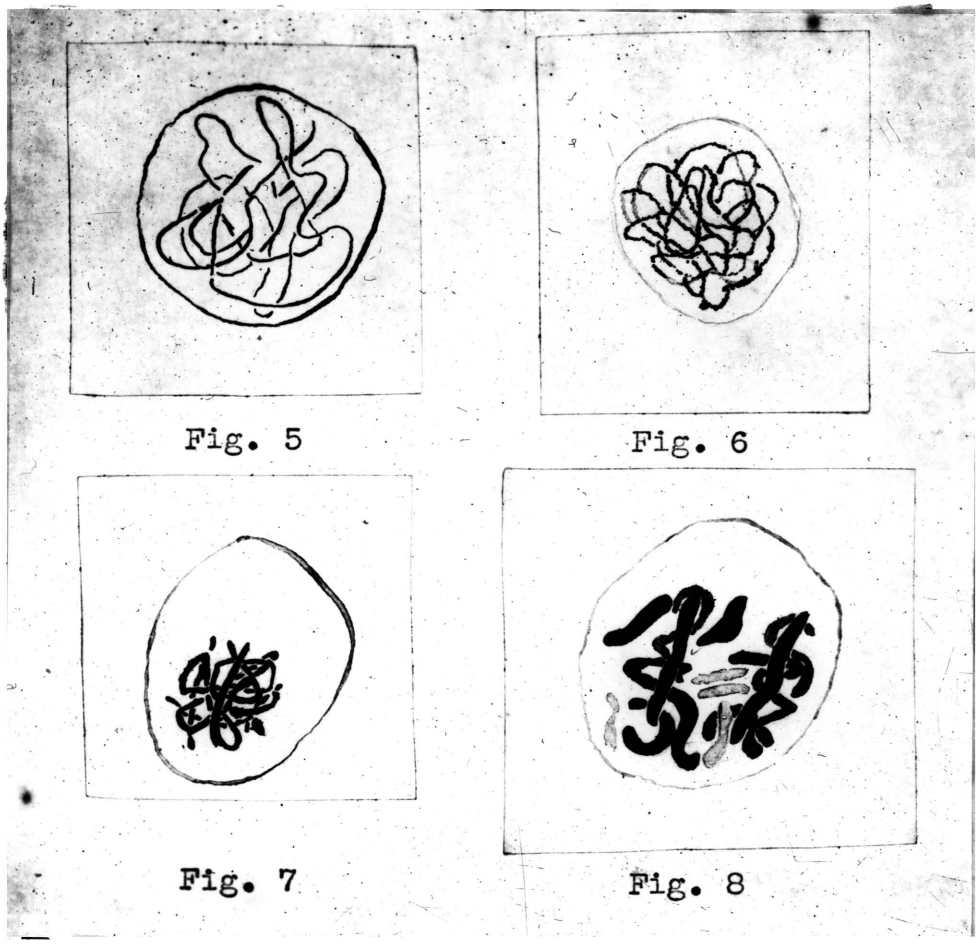

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 5. Camera lucida drawing of the leptotene stage of oogenesis.

Fig. 6. Camera lucida drawing of the zygotene stage of oogenesis.

Fig. 7. Camera lucida drawing of the pachytene stage of oogenesis.

Fig. 8. Camera lucida drawing of a growth stage in the diplotene showing the chromosomes scattered throughout the cell.

PLATE III

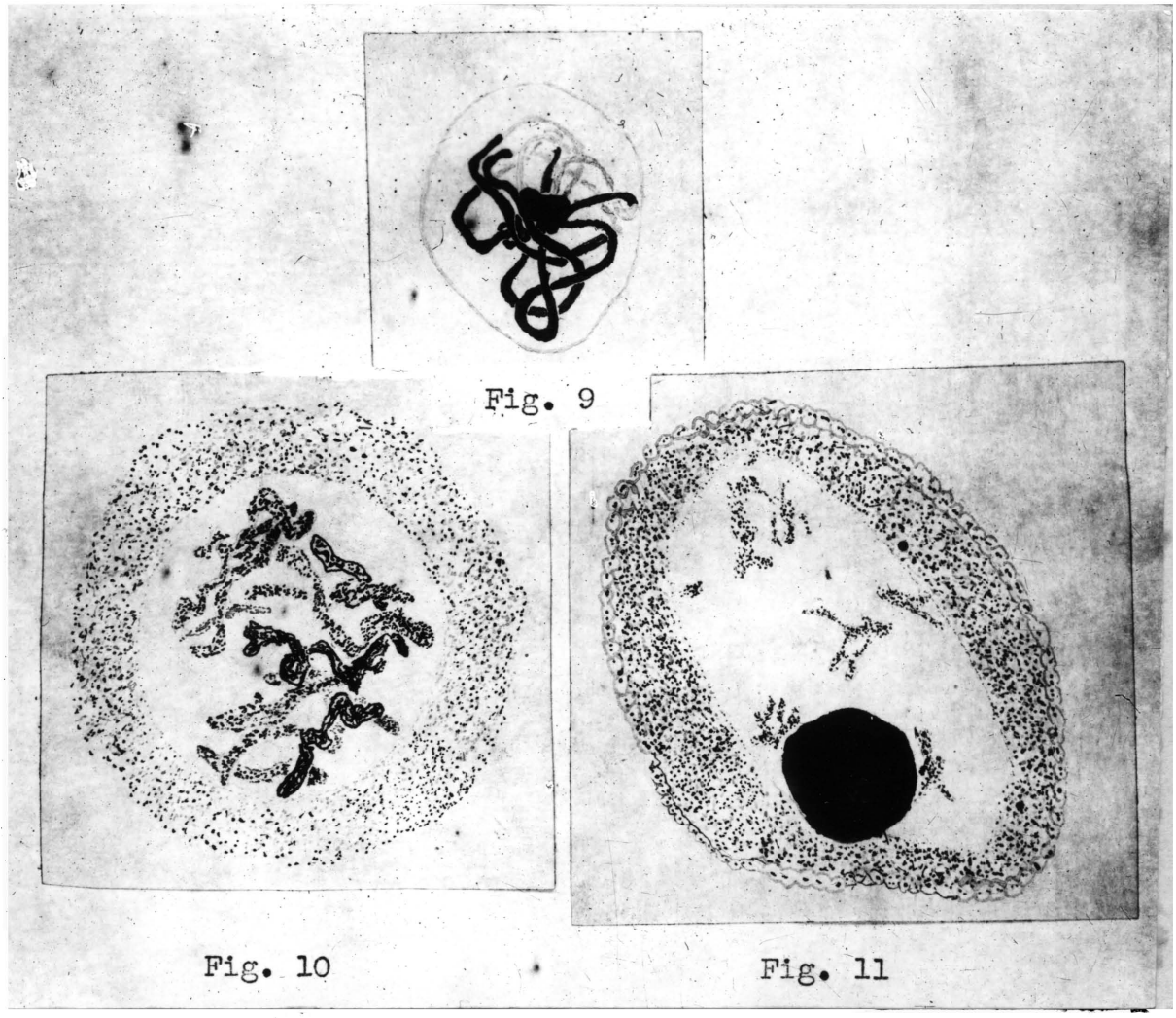

Fig. 9

Fig. 10

Fig. 11

Fig. 9. Camera lucida drawing of the synizesis of oogenesis.

Fig. 10. Camera lucida drawing of the vacuolated chromosome condition or diplotene of the second growth period of oogenesis.

Fig. 11. Camera lucida drawing of the germinal vesicle stage of oogenesis.

PLATE IV

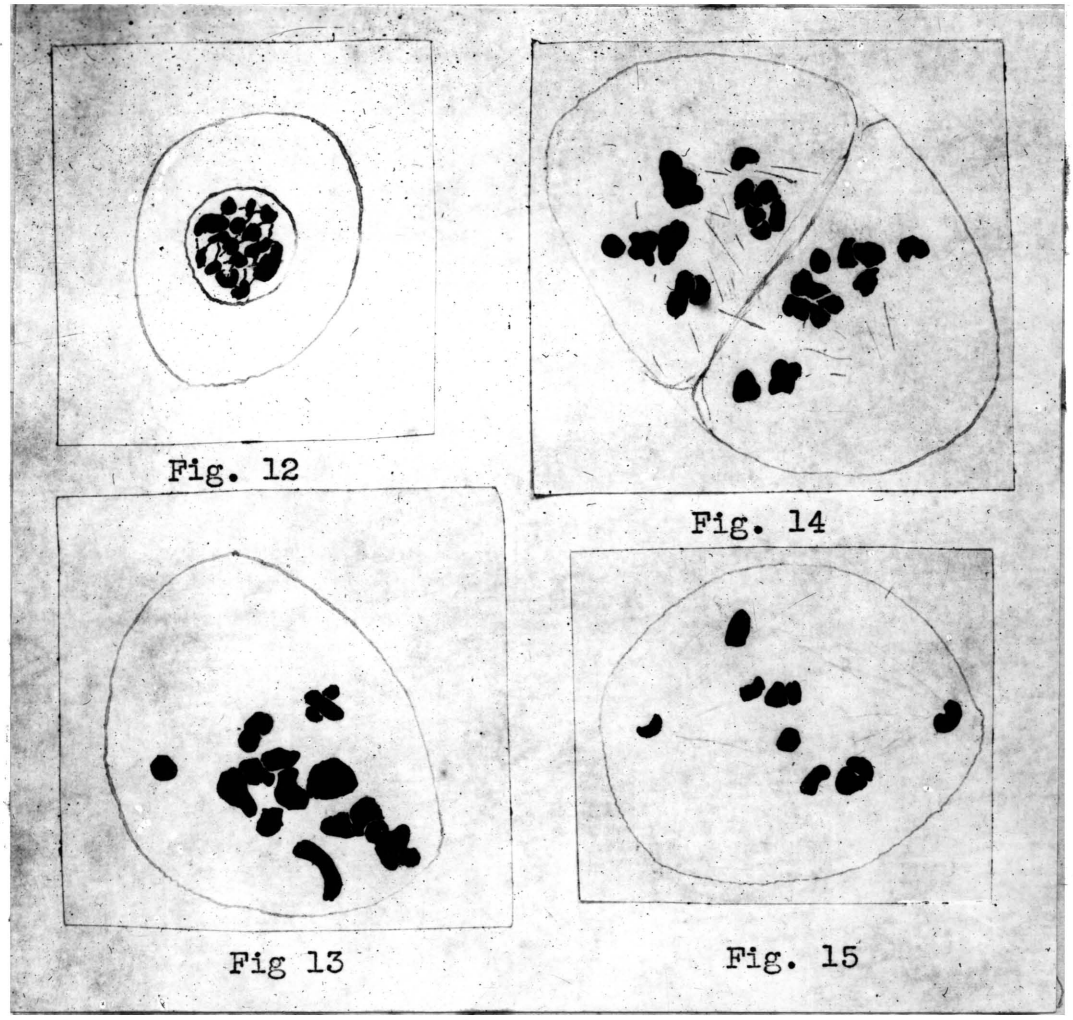

Fig. 12. Camera lucida drawing of a polar oogonium.

Fig. 13. Camera lucida drawing of the diakinesis of spermatogenesis. Note the sex chromosomes.

Fig. 14. Camera lucida drawing of two cells showing the tetrads lined up on the spindle preparatory to the reduction division in spermatogenesis. Note the sex chromosomes.

Fig. 15. Camera lucida drawing of a secondary spermatocyte preparatory to division to form the spermatid. Note the advance of sex chromosomes.

PLATE V

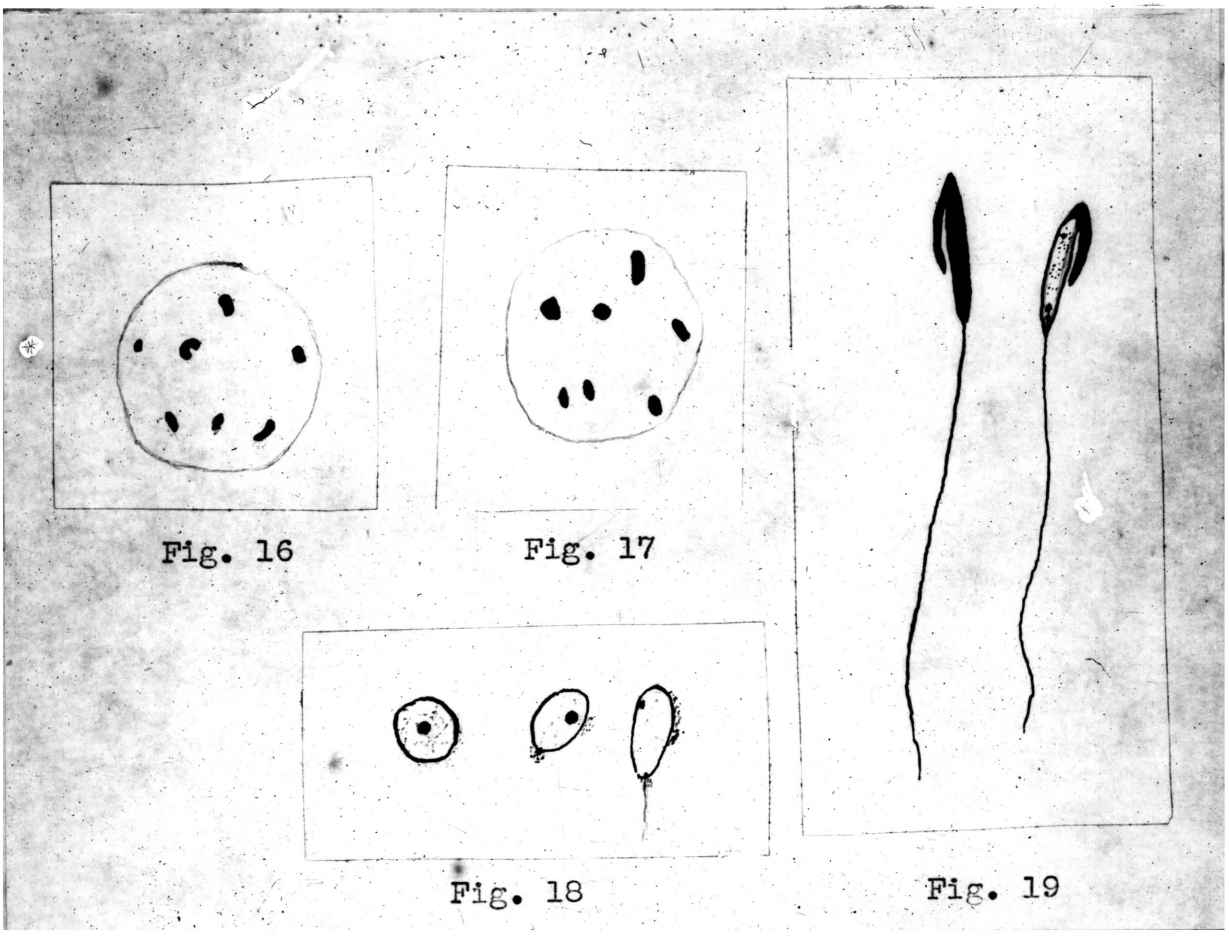

Fig. 16. Camera lucida drawing of a secondary spermatocyte showing the X chromosome.

Fig. 17. Camera lucida drawing of a secondary spermatocyte showing the Y chromosome.

Fig. 18. Camera lucida drawings of representative stages in the metamorphosis of the spermatid.

Fig. 19. Camera lucida drawings of mature spermatozoa, the first is darkly stained, and the second is stained lightly.

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