SPERMATOGENESIS IN LEPIDOPTERA.

MARGARET HARRIS COOK, PH.D

CONTENTS.

INTRODUCTION.

Ever since Henking's work on the spermatogenesis of *Pyrrhocoris apterus* (1891) it has been known that in one maturation division one chromosome may go undivided into one of the daughter cells, so giving two classes of spermatids. The significance of this fact was not recognized until ten years later, when McClung (1901) advanced the purely theoretical view that this dimorphism of the spermatozoa bore a direct relation to the determination of sex, and suggested that the spermatozoa containing the extra chromosome, which was called by him the accessory chromosome, were the male determinants. In 1905 Stevens for Coleoptera and Wilson for Hemiptera showed, by comparing the number of chromosomes in the spermatocyte and oöcyte, that the accessory chromosome had its homologue in the egg and hence was the female and not the male determinant. That this marked difference in the behavior of the accessory chromosomes from the other chromosomes in one of the maturation divisions, together with the new interpretation of its

function, might throw light on the chromatin substance and its bearing on heredity seemed more probable than ever before, especially if it could be shown that this held true for all classes of animals. In summarizing the work on this subject, McClung (1902) says the accessory chromosome has been found in all insects so far studied, is present in different members of Orthoptera, and, in examining material from Hemiptera, Neuroptera, Coleoptera, and Lepidoptera, this body was found. Miss Wallace has found it in Arachnida and the probability of its general occurrence in Arthropods is thus largely increased. Some hurried examination of vertebrate spermatocytes lead McClung to believe that the accessory chromosome is likewise present among vertebrates and that it will probably be found to be universal.

Except this reference of McClung's to the accessory in Lepidoptera, no other mention of it was found; and, since no recent detailed examination of the spermatogenesis of Lepidoptera had been undertaken, it seemed advisable to examine members of this group. Accordingly, in September, 1905, at Dr. Conklin's suggestion, I began to collect material with the purpose of studying the accessory chromosome in Lepidoptera. Testes were fixed and cut from a number of genera and species, but in most cases were found unsatisfactory. *Philosamia cynthia* was particularly clear and was chosen as a basis for comparative work. Owing to the publication of Dederer (1907) on this species, I shall refer to my own work only when my results differ from hers.

This paper contains, aside from references to *Philosamia cynthia*, a study of the spermatogenesis of *Callosamia promethea*, *Telea polyphemus*, *Automeris io*, *Samia cecropia*, and *Acronycta* (sp.?) as well as a few comparisons with *Danais archippus* and *Papilio cresphontes*. I am indebted to Prof. Calvert, of the University of Pennsylvania, and to Dr. Skinner, of the Academy of Natural Sciences, for aid in the identification of the species here described. The work was done in the Biological Laboratory of the University of Pennsylvania under the direction of Prof. Edwin G. Conklin. I am glad of this opportunity to express my feeling of gratitude to Prof. Conklin for his help and encouragement throughout the years of my work as well as for his suggestions and for criticisms of this paper.

I. Previous Work.

The published works upon Spermatogenesis in Lepidoptera may be divided into two classes: the earlier works, including those of Carnoy (1884), Platner (1889), Cholodkovsky (1894), Verson (1894), Erlanger (1896), La Valette St. George (1897), Henneguy (1891), Meves (1890),

and Toyama (1903); the later, those of Munson (1907), Stevens (1906), and Dederer (1907). The earlier workers concerned themselves principally with the origin of the sex cells and the formation of the spermatozoa, with especial reference to achromatic structures. Thus Cholodkovsky finds in the closed flask-shaped end of the testis tube of *Laphria* a large cell from which the cells of the testis arise; and the same subject has been studied by Verson, La Valette St. George and Toyama for *Bombyx mori*. Of all the earlier workers it is perhaps to La Valette St. George, more than to any student of this group, that we are most indebted, for to him we owe our present nomenclature. In 1884 Carnoy, studying the larval testes of two moths, *Chelonia caja* and *Arctia fuliginosa*, made the observation, confirmed for the species here studied, that all the cells in the same cyst are in the same stage of development and he figures and describes a few steps in the development of the first spermatocytic division. In *C. caja* the twenty-four rodlike chromosomes divide longitudinally and move to opposite poles, while in *A. fuliginosa* the chromosomes in a side view are seen upon a spindle as dumb-bells with a transverse constriction. An equatorial plate shows twenty-eight chromosomes so arranged that the twenty peripheral chromosomes surround the eight interior ones. Carnoy's observations on this group are very limited, owing to the fact that he found his material unsatisfactory for studying the reconstructive stages of the nucleus. Platner was the first to describe the whole course of development of spermatogenesis for Lepidoptera. He described the thirty chromosomes in the first spermatocytic division of *Pygœra* and *Sphinx* as short rods with transverse constrictions, and since the rods in the mitotic figure are arranged parallel to the spindle axis, this constriction marks the first plane of division. He confused the acrosome and centrosome, yet confirmed Bütschli's observations by showing that the "large mitosome," Bütschli's "Nebenkern", functions in the formation of the tail of the spermatid. His work, like that of Erlanger (1896) and LaValette St. George (1897), deals largely with the achromatic structures of the nucleus. Of special interest in the light of recent work is Platner's account of the nucleolus, which he describes as one or two rather large and deeply staining bodies which are spherical in shape and eccentrically placed. It seems very probable that these are comparable to the equal pair of idiochromosomes figured for the forms described in the present study.

Toyama (1902; his second paper, 1903, I have been unable to find) describes the whole spermatogenic cycle for *Bombyx*. He begins with a discussion of the formation of the early spermatogonia in the per-

ipheral end of the testis around a large cell which he considers a follicle cell, in opposition to Verson's (1894) idea that it is a large spermatogonium, which by mitotic division gives rise to all the spermatogonia. La Valette St. George (1897), also working on *Bombyx*, failed to confirm Verson. Toyama finds twenty-six to twenty-eight chromosomes in the spermatogonia. These split longitudinally before the formation of the spindle, so that they appear in the prophase of the first spermatocyte as ring-shaped bodies which are each made up of four chromosomes. According to Toyama the first division of these chromosomes is transverse; the second is not a true division but a separation of whole chromosomes, fourteen passing into each cell. During the spindle stage a persistent nucleolus is found which shows no change in the resting stage of the first spermatocytes and is seen to consist of small chromatin granules imbedded in a less dense matrix. This nucleolus is later pushed into the cytoplasm. Meves (1897) working on six species of Lepidoptera, confined himself largely to cytoplasmic structures. He assigns a different origin to the Nebenkern from that of Bütschli and Platner and makes the interesting observation, for the first time, that an axial filament grows out of the centrosome of the resting spermatocyte. Henneguy (1897) confirms this observation of Meves for *Bombyx mori* and *Hyponomenta cognatella*.

Of the more recent contributions, Munson's (1906) on *Papilio rutulus* is chiefly devoted to cytoplasmic structures and is hard to bring into relation with the observations of modern workers, since it shows no contraction stage of the chromatin and no synapsis, "unless a temporary conjugation may be considered to take place when the twenty-eight chromosomes are arranged in a line of seven, four deep." These "tetrads" break up into fourteen dyads. The first maturation division is equational, the second, like that described by Toyama, is not a true division, but a sorting of chromosomes, fourteen going to one pole, fourteen to the other. In the work of Stevens and Dederer special attention is given to the chromatic elements. Stevens (1906) figures and briefly describes the spermatogenesis of *Cacœcia* and *Euvanessa*. In both species there are thirty chromosomes in the first and second spermatocytes and in both there is one large chromosome, which corresponds to the two-lobed body in the growth stage; this body stains differentially in gentian-violet and can be traced through synizesis, synapsis, growth stages, and both first and second maturation divisions, and is interpreted as an equal pair of idiochromosomes comparable to those found by Wilson (1905, *b*) in *Nezara*. Dederer (1907) studied the spermatogenesis of *P. cynthia*. Of the thirteen chromosomes (reduced

number) one shows marked individuality throughout; it is always in close connection with the plasmasome, stains deeply when the other chromosomes have lost their staining properties, and in early prophase is seen to take a form similar to the other chromosomes. Dederer, like Stevens, interprets this chromosome, as a pair of equal idiochromosomes of the *Nezara* type.

II. Material and Methods.

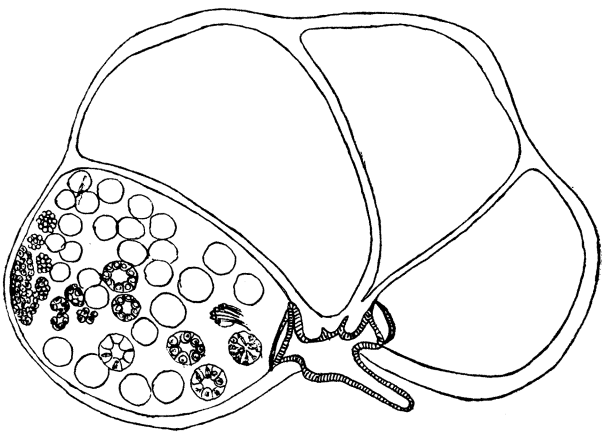
My material was largely collected in the vicinity of Philadelphia, and was fixed at intervals throughout the pupal stage. The ventral wall of the abdomen was cut along its length and pinned back; after removing the intestines, the fixing fluid was immediately injected into the body so that the testes were fixed during dissection. Of a number of solutions used, Flemming's strong solution, Hermann's platino-aceto osmic, and Gilson's mercuro-nitric gave the best results. Material fixed in Flemming for from six to twelve hours, washed the same length of time in running water, and preserved in various percentages of alcohol, was most satisfactory: here both chromatic and achromatic structures were sharply defined, and the shrinkage which a longer fixation so often produces was avoided. Preparations which had been so fixed and run through the alcohols were then cleared in xylol and embedded in hard paraffin with a melting point of 55° C. Sections were cut from three to nine microns thick. Various stains were used: The best cytological results were obtained with Heidenhain's hæmatoxylin followed by orange-G; this stain gave an almost perfect definition and was used for all general and for most of the detailed work. Thionin, Delafield's hæmatoxylin, safranin and light green, Hermann's safranin-gentian-violet, Auerbach's and Biondi-Erlich's stains were used for micro-chemical tests, but with at best indifferent success. The greatest difficulty encountered in technique has been in finding a stain which will clearly differentiate basichromatin from oxychromatin. In the early spring of 1907 I began to make smear preparations by spreading bits of testes upon a slide with dissecting needles, and after drying, staining them in Bismarck Brown for twenty-four hours as described by Foot-Strobell (1905). These proved very helpful. The cells stretched in drying and were several times larger than those in sections, so that they were especially useful in studying spireme stages and in counting chromosomes in cell plates. Their disadvantage lies chiefly in the lack of the sequence of stages so clear in sections, but when studied in connection with sections and particularly when the number of chromosomes is small, they have been invaluable.

III. Observations.

To the family of Saturnids belong those moths which are silk weavers *par excellence* in the caterpillar state. Among our most common native species are *Callosamia promethea* and *Samia cecropia*, which emerge from the pupa case in May or June. Much has been written of the keenness of the mating instinct in *promethea* and my own observations confirm these accounts. The moths mate and lay eggs which hatch in from eleven to fourteen days and the caterpillar pupates in from five and a half to eight weeks, depending upon its supply of food. The pupæ of *C. promethea* are very like those of *P. cynthia* and the two may be easily confused. This is especially true since the caterpillar of *P. cynthia* will live upon the same food plants as that of *C. promethea*, but in this case it is smaller than usual and weaves a cocoon just like that of *C. promethea*. The number of chromosomes in an equatorial plate differs in the two and it was sometimes necessary to use this means of identifying these two species.

The testes inLepidoptera originate as paired glands. They are situated under and on each side of the intestine in the region of the sixth segment. In moths of the family Saturnidæ these organs are paired and develop in size until just before the moth emerges, when the ripe spermatozoa are discharged into the greatly enlarged vas deferens and the testes become smaller, shrivelled, and translucent. In *Danais archippus*, *Papilio cresphontes*, and in *Acronycta* (sp.?) the testes, which are paired in the larval stage, during the pupal stage become closely applied to each other along the midline and form a single spheroidal body, colored almost royal purple in *D. archippus* due to the pigment of the surrounding epithelial coat. In *D. archippus* (summer brood) the testes contained developed spermatozoa six days after pupation, the time of pupal development being from eleven to fourteen days. In moths the rate of development varies with the species. Some *S. cecropia* and *A. io* kept in the laboratory became imagoes in January, while *C. promethea* under similar conditions shows much greater retardation. *Promethea* fixed in February showed a few first maturation divisions and then came a long period when from March to May development of the spermatozoa did not proceed farther than the spermatids. The testes of the saturnids are kidney-shaped, tinged the faintest yellow and divided into four lobes; they are surrounded by a layer of connecting tissue, which forms partitions between the lobes and between the cysts. The cells are arranged in the order of their development, from the periphery inward: the extreme anterior end of the testis until late in development is occupied by the primordial germ cells—the sperma-

togonia, either singly or in groups, but not yet surrounded by a membrane. No early larval material was obtained, so the origin of the spermatogonia, whether from a "Verson cell," a metamorphosed spermatogonium, or arising around a supporting follicle cell, cannot be discussed. The primordial germ cells divide and redivide until a certain number of daughter cells is formed, usually from sixteen to twenty-four. These are then grouped into cysts surrounded by connective tissue and containing cells in about the same stage of development. Between this region of the spermatogonia and the spermatocyte of the growth period are found all stages of synizesis and synapsis.



Throughout the testes, but especially where spermatogonia are changing into spermatocytes, whole cysts of degenerating cells are found; in these the chromatin is gathered into one large or several small, compact, deeply staining granules, while the cytoplasm is granular and stringy. In Auerbach's and other differential stains these granules stain like active chromatin, showing that a chemical change is taking place in them. Though these degenerating cysts are far more common in this region than elsewhere, yet they are found throughout the testes, especially where spermatids are forming. Paulmier (1899) figured and described just such degenerating cells for *Anasa tristis*; though here they are found only after the spermatogonic and before the spermatocytic stages: their appearance in this region of special growth caused Paulmier to interpret them as "food cells." Munson (1906) suggests that a cause for disintegration may be found in the failure of the chromatin to secrete karyolymph. Dederer's (1907)

observation of the division in autumn of a few first spermatocytes of *P. cynthia* and her suggestion that these degenerating cysts of cells can be traced to the degeneration of those cells which have undergone precocious maturation seems an excellent explanation of their origin.

Next to these synapsis and synizesis stages and arranged in some sort of sequence from the periphery to the center of the cysts are found spermatocytes in various stages of growth; primary spermatocytes, secondary spermatocytes, spermatids, and developing and developed spermatozoa.

Callosamia promethea.

1. *Spermatogonia.*—The spermatogonia of *promethea* are small oval cells which in their earlier stages are crowded together and closely pressed against the walls of the testes. Secondary spermatogonia are somewhat larger and extend further inward so that they are easily studied. In what Wilson (1902) says has been falsely characterized as "the resting stage", the nuclei are spherical and faintly staining and fill by far the greater part of the cell; within the nucleus is found an irregular meshwork of linin threads upon which chromatin granules are scattered. In the early spermatogonial cells (Pl. XXII, figs. 1, 2 and 3) there are found chromatic masses or "net knots": they are spherical and resemble plasmosomes in which chromatin granules are entangled. These granules are of the same size as those scattered throughout the nucleus, and that they are simply an aggregation of these is suggested by the fact that in later stages, when the granules are more widely scattered through the nucleus, this net knot, after decreasing in size and in the number of contained granules, finally disappears (fig. 4). The basichromatin granules now stain more deeply and become aggregated along the linin to form a spireme. It has been impossible in *C. promethea* to distinguish any structure in this spireme, though in smear preparations of *P. cynthia* it is clearly seen to be composed of twenty-six pairs of chromosomes lying side by side and each joined to the other by linin threads. The significance of this will be discussed later. The chromatin of the spireme condenses, and in equatorial plates of the last spermatogonial division thirty-eight small, densely stained, and compact chromosomes may be made out (fig 6). In smear preparations this plate is easily seen, and among the chromosomes one pair is very plainly smaller than all the others; but even here, where the size of the plate is enlarged many times by the method of fixation, it is still too small to offer much opportunity for studying size relations.

In preparation for the last spermatogonial division the chromosomes become regularly arranged upon the spindles, divide equally and **are**

drawn towards the poles, the cell lengthens and with it the connecting fibres. At the point at which the new cell wall will be laid down a zwischenkörper of four small granules is to be seen (fig. 9). It was just at this stage of telophase that Montgomery (1900) was able to demonstrate that synapsis took place by an end-to-end union of homologous pairs of chromosomes; in *C. promethea* the chromosomes were often distinguishable as somewhat feathery bodies with linin connections, but they were so closely crowded, due to the small size of the cell and large number of chromosomes, that it was impossible to follow the process. After the separation of the chromosomes the centrosomes divide and the nuclear membrane is reconstructed to form the cells of the primary spermatocyte.

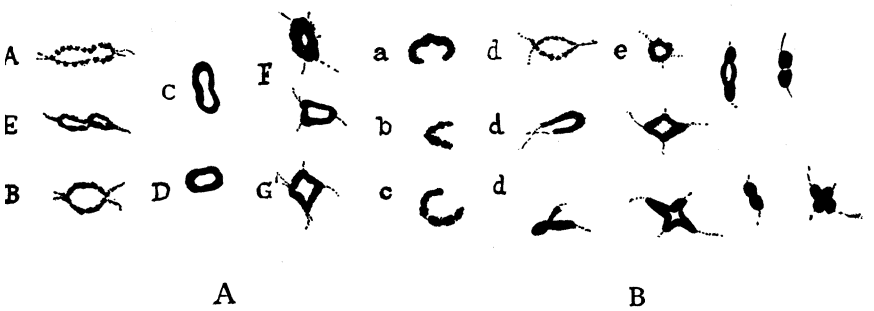
2. *Growth Period of the Primary Spermatocyte.*—It is during this period, when the cells grow but do not divide, that the chromatin undergoes marked changes: it first forms a long spireme which is seen to be looped on one side of the nucleus; as the looping proceeds, the chromatin mass stains more deeply until it is impossible in ordinary preparations to make out anything of the spireme arrangement (fig. 12). This is the "synizesis" of McClung or "the contraction stage" of Wilson. Since similar stages have been described by Wilson, Stevens, and a number of workers on insect spermatogenesis, and since similar stages appear in smear preparations, there seems little doubt but that this contraction stage plays a perfectly normal part in the constructive life of the cell and is not an artifact, as argued by McClung (1900). The number of cells showing different stages in synizesis varies with the period of development: in young pupæ they may occupy almost half the testes, while in later stages relatively few cells may be found which show this condition. What takes place during this period of intense staining and contraction of the chromatin it has been impossible to make out; for even when much destained little can be seen. Some help has been found in smear preparations where the long thin thread can be clearly seen to be formed of chromatin granules with linin connections (fig. 13); though this stains much less deeply than in sections, so that somewhat of its structure can be made out, yet it is never definite enough to enable one to follow the steps of contraction nor to determine whether conjugation of the chromosomes takes place during this stage. The chromatin comes out of the contraction stage by an unwinding of the condensed thread; in a few cases this could be traced as a continuous spireme (fig. 19), though usually it is made up of a small number of loops which at first only partly fill the nucleus: this corresponds to "stage e" or "early post synapsis" of Wilson (1905).

In the next stage, or late post-synapsis (figs. 20 and 21), the threads are somewhat contracted but more widely spread throughout the nucleus. In smear preparations (figs. 19, 20 and 21) the spireme of both early and late post-synapsis shows a longitudinal split which corresponds to a similar split in the last spermatogonial spireme (as seen in *cynthia*). The spireme now segments into the reduced number of chromosomes (fig 22): in smear preparations it may still show the longitudinal split of the late synapsis stage; in sections, where there is always a greater concentration of chromatin granules, the spireme is shown segmented into loops which have their middle point marked either by a thinner part or by a slight knob-like projection. This marks the plane of the first division (fig. 22). During the growth stage the spermatocyte greatly increases in size, while its basi-chromatin almost completely loses its staining property and becomes loose and indefinite in structure. This is true only in part, for in smear preparations, in which the chromatin segments stain less deeply but never entirely lose their identity (fig. 26), the chromosomes are seen as faintly staining bodies made up of chromomeres joined by linin threads. It is at this period that, for the first time, a spheroidal, darkly staining body appears and stands out in sharp contrast to the faintly stained nucleus; it is usually eccentrically placed, and is often seen dividing so that it may appear as two separate bodies or as a dumbbell (fig. 23). Its appearance, behavior, and staining reaction suggest its similarity to the accessory chromosome of McClung, the chromatin nucleus of Montgomery (1901), the heterotropic chromosome or idiochromosome of Wilson, and the odd chromosome of Stevens; while its frequent dyad nature during the growth period, indicating its bivalence, and its subsequent behavior, relate it to the third type described by Wilson, in which the idiochromosomes are of equal size. Following the terminology of Wilson, I shall call this body the idiochromosome.

3. *Prophase of First Maturation Division.*—In preparation for the first maturation division the chromatin again assumes its staining property and definite groups of chromatin granules appear upon the spireme, the nineteen aggregations of basi-chromatin which have been distinguishable throughout the growth stage in smear preparations now become more clearly defined, the longitudinal split of the spireme is still seen and each chromosome is joined to the other by a continuous linin thread (fig. 26). A similar condition is seen in sections where each chromosome shows a longitudinal split, while some of the chromosomes are beginning to join to form rings (fig. 27). In a little later stage the segments have opened into ring-like granules (figs. 32 and 28) and

exactly correspond at this time to the ring-shaped chromosomes figured by vom Rath (1892) for *Gryllotalpa*. Blackman (1905) considers the first maturation division of *Scolopendra* a longitudinal one because "the longitudinal division of the chromatin segments is the first which occurs in prophase"; but though in *promethea* the longitudinal division is present from post-synapsis, yet this is clearly the plane of the second maturation division and must be explained as a precocious splitting.

Constrictions such as are seen in figs. 22 and 26 can be easily traced to such stages as are seen in figs. 28, 30 and 31. By concentration small thicker rings are formed, until, by continued thickening, the central space becomes more and more reduced and in most cases the longitudinal split is entirely or almost entirely concealed. It may be retained as a narrow slit between the two ends of a dumbbell or a rounded or diamond-shaped space in the center of an occasional tetrad (figs. 37 and 38).



A B

The changes in form which the chromosomes undergo during prophase are shown in text figs. A and B.

In early prophase the chromosomes appear first as granular aggregations, as at A and E; these aggregations next open wide to form granular rings B; then by condensation the rings become much thicker and the granular appearance is lost. Rings may be lengthened, C, and show slight constrictions which mark the plane of the first division, or may be clearly rounded, D; F and G shows an approach to tetrads. Complete condensation is rarely seen in smear preparations. a, b, c, are the earliest stages shown in sections. These are made up of more of less condensed granules with no indication of a longitudinal split, but a slight constriction is usually seen as in a and b; and though no split is seen, yet stages like d and e make it necessary to assume such a stage. The granules are single and the ring of about one-half the thickness of the loop, and the ends which overlap in d become joined in

e. Condensation now takes place into dumbbells and tetrads and the longitudinal and transverse planes of division can be clearly made out.

4. *Maturation Divisions.*—The chromosomes formed by the condensation of rings now become arranged upon the fibres so that a side view of the spindle during metaphase shows the chromosomes as symmetrical dyads which are so placed that the first division, which is always equal, may be seen to be transverse, and, if conjugation has taken place as I believe by an end-to-end union of the chromosomes, division is reducing (Pl. XXIII, figs. 42, 43). An equatorial plate during metaphase shows nineteen chromosomes (Pl. XXIII. fig. 40). While the chromosomes are in metaphase the centrosomes divide and each becomes enclosed by a small centrosphere; as division proceeds the astral rays increase in length until they seem to press against the wall of the cell, a constriction is seen in the cytoplasm and the zwischenkörper is formed at this point (fig. 44). During late anaphase the chromosomes become so crowded as to lose their separate outlines (fig. 45), but these become distinguishable in the prophase of the second maturation which is rapidly passed over (fig. 46).

The spindle of the second maturation division is quickly formed and the chromosomes become arranged upon it as dyads (fig. 50). The second maturation spindle in both methods of fixation can be distinguished from the first by its smaller size and by the smaller size of the chromosomes. Division, like that of the first maturation, is equal, and the chromosomes may be seen in anaphase as separate, spherical bodies (fig. 51), while in telophase they are a densely staining mass about which a nuclear membrane is forming (figs. 53 and 54). The second division of the chromosomes is longitudinal and may be traced back to the longitudinal split of the spireme. The centrosomes and their accompanying structures show much the same behavior in the second as in the first division; the cells increase greatly in length and a constriction, which is marked by the zwischenkörper, appears at the equator.

Since there is no unequal division of chromatin material in either the first or second maturation divisions, it is clear that there is no "accessory chromosome" and hence no visible dimorphism of the spermatozoa; there are, however, reasons for believing that the peculiarity of behavior of one of the bivalents during the growth stage classes it with *Nezara* as an equal pair of idiochromosomes.

5. *The Idiochromosome.*—While the other chromosomes have been undergoing these changes of form, the idiochromosome described for the growth stage as a single or double body (figs. 23–29), which reacted

to basi-chromatin stains, has also changed in shape and apparently in structure. What during the growth stage appeared as a homogeneous mass shows as prophase advances a clearer plasmosome and a darker chromatin part. In smear preparations, when all the chromosomes can be seen at one time, it becomes perfectly clear that this idiochromosome forms one of the nineteen chromosomes. Fig. 39 shows this to be true even into late prophase. This is a smear preparation in which the plasmosome of the same size as the chromosomes may be recognized as a clearer body with a central chromatin band. In early prophase in both smears and sections, when the spireme is splitting or opening into rays, this body may assume a ring shape (fig. 27), showing a lighter central space around which is arranged the chromatin. Because of its close connection with the plasmosome the idiochromosome always appears larger in these stages than any of the other elements, but with its condensation into a ring or dumbbell-shaped chromosome this size relation is lost, and after its dissociation from the plasmosome I was unable to distinguish it from the other chromosomes. Although this idiochromosome is first seen in *C. promethea* after the late post-synapsis when the chromatin has lost its staining property, yet its subsequent history makes it seem more than probable that it was present from the earliest stages: whether the net knots of the spermatogonia bear any relation to this or whether the idiochromosome has been separated from the rest of the chromatin during synizesis must remain conjectural. Its condensed condition during the growth stage and frequent early division while the rest of the chromatin is passing through the usual series of changes before entering upon prophase, as well as its close relationship with the plasmosome, point to the fact that, certainly in structure and possibly in function, this idiochromosome shows peculiarities which separate it from the other chromosomes.

6. *Metamorphosis of the Spermatids.*—The two spermatids which arise as a result of the second spermatocytic division are elongated cells with a rather small nucleus whose chromatin is in the form of granules. In the telophase of the second maturation division, before the nuclear membrane is complete, the chromosomes are more or less condensed and surrounded by the remains of the spindle fibres of the last spermatocytic division (fig. 54); this spindle fibre material persists and is later traced to the nebenkern. The nucleus assumes an eccentric position so that it lies very near the wall of the cyst, and as the nuclear membrane is formed, the chromatin becomes more scattered throughout the nucleus and the material of the spindle fibre, which

at first was irregularly arranged, is now collected into a spherical mass at the proximal pole of the nucleus to form the beginning of the nebenkern (fig. 56). The centrosomes in an archoplasmic mass still retain their position at the distal pole of the nucleus, and from the more distal one is seen to grow out a flagellum which corresponds to similar structures to be described for both first and second spermatocytes and interpreted as a precocious axial filament (figs. 55 and 56). As the spermatid develops the centrosomes may be seen to migrate until they assume their ultimate position at the posterior side of the spermhead and the archoplasm or idiozome which surrounds the centrosomes migrates with them (figs 57–59). After the centrosomes have moved around to their final position at the proximal pole of the nucleus, the idiozome is seen as a small body, clearer than the nebenkern and lying beside it (fig. 60), and at this time, in the nucleus of each spermatid, a round darkly staining body is to be seen (figs 56–61). Since there is no evidence of an unequal division in either first or second maturations and since this body is found in most of the spermatids (its absence in some being explained by oblique cutting), there seems no likelihood of its bearing any relation to the accessory chromosome described by so many workers on insect spermatogenesis. I conclude that it is a new formation, and that it is comparable to a similar body described as "chromatin nucleolus" by Stevens (1906) for Coleoptera and by Boring (1907) for Hemiptera. From the distal centrosome, which now lies just beside the nucleus, the axial fibre continues to grow; and as this grows through the center of the nebenkern and cell cytoplasm both elongate, the former to form the inner, and the latter the outer tail envelope.

7. *Centrosomes.*—In the earlier "rest stages" of the spermatogonia the centrosomes, which could be followed through every subsequent step of development, were not visible; but the amount of cytoplasm is so small and the cells so crowded that they might easily be overlooked, and since they are to be seen in all spermatogonic divisions of both the primary and secondary spermatogonia they may be assumed to be present even though not seen except when the cells are actively dividing. Just before the formation of the last spermatogonial spireme (figs. 4 and 5), when the chromatin is scattered through the nucleus as separate granules, two minute centrosomes situated near the nucleus are seen to divide and move toward opposite poles: the nuclear membrane disappears and the mitotic figure is formed as usual (figs. 7 and 8). After the division of the chromosomes the centrosomes divide and may be seen surrounded by a mass of archoplasm (fig. 10).

From this stage the centrosomes which have been followed through every step of development show unfailing persistence and regularity in movement, in form, and in division. This observation, like that of Conklin (1902) for *Crepidula* and Paulmier for *Anasa*, supports the view that the centrosome is entitled to the rank of a permanent cell organ.

In the succeeding growth stage the centrosomes surrounded by faint astral rays are seen to separate; then they divide: one pair moves towards the nulcear membrane, the other pair lies upon the cell wall (fig. 25). In preparations which have been somewhat deeply stained cilia of considerable length are seen to have grown out from each granule and extend into the lumen of the cyst (figs. 24, 25 and 29); these correspond to the ciliated centrosomes figured by Meves for *Pygœra* (1897) and by Henneguy for *Bombyx* (1898), differing only from those shown by Meves in the shapes of the centrosomes, which in *Pygœra* are described as hooked or V-shaped bodies. In *C. promethea* these are plainly small dumbbell-shaped structures and are exactly like the centrosomes described by most workers, differing only in the possession of the flagellum.

While the chromosomes are passing through the prophase of the first maturation division, the paired centrosomes, enclosed by a small centrosphere, move to opposite sides of the nucleus where they appear as dumbbell-shaped bodies surrounded by a clear archoplasmic zone and short radiating fibres (figs. 31 and 32). As the nuclear membrane disintegrates, the astral figure increases in size, its fibres extending outward into the cytoplasm and inward towards the middle of the cell, and as division proceeds the astral rays increase in length until they seem to press against the wall of the cell. I have never observed a flagellum going from the centrosome at this stage, though such has been figured and described by Henneguy. After completion of the metaphase the centrosomes divide and appear in the telophase of the first maturation division as two separate bodies, each surrounded by an archoplasmic mass; the centrosomes migrate to opposite sides of the nucleus in an axis at right angles to the first maturation spindle. Just at this time a flagellum is seen to grow out of one of the centrosomes (fig. 48), this flagellum, though somewhat longer, is similar to those described for the first spermatocytes.

One other structure in the cytoplasm which is of interest is the "chromatin granule" first seen during the growth period (fig. 25). These granules, either single or dividing, stain like chromatin, are surrounded by a clear zone, and are traceable through succeeding stages;

that they may have some functional significance seems probable and this will be discussed under the development of the spermatid.

Telea polyphemus.

Telea polyphemus, which belongs to the sub-family Saturinæ, is found in its larval stage feeding upon oak and other shade trees. After the cocoon is made, in part from the folded leaves of its food plant, it falls to the ground. Material was collected from about Bellefonte, Pa., and from Newark, N. J., through the kindness of Mr. Herman H. Brehme. Development in *T. polyphemus* is somewhat more rapid than in *promethea*, and shows a greater dependence upon temperature conditions.

Spermatogonia which are found in the periphery of the testes show always an eccentrically placed mass formed by a plasmosome in which is embedded a number of darkly staining granules (fig. 62). The last spermatogonial division is preceded by the formation of a thick spireme which segments into a large number of chromosomes, probably sixty, though the number is so great and the chromosomes so massed that it is impossible to be sure that this count is correct (Pl. XXIV, fig. 66). A side view of the spindle shows the dumbbell-shaped chromosomes arranged upon the spindle fibres (fig. 67); these divide symmetrically and are seen in telophase as feathery chromosomes arranged upon linin threads (fig. 69). It is possible that a synapsis takes place at this time, but, owing to the large number of chromosomes, I have been unable to observe it. The chromatin now forms a long slender spireme which in both smears and sections is seen to be made up of granules (fig. 70). This spireme gradually becomes looped (fig. 71) until concentration is complete and all the chromatin lies in a darkly stained mass against one side of the nucleus (fig. 73); the chromatin then passes out of this contraction stage by a loosening of the loops (figs. 74 and 75), which stretch out into the nucleus and in late post-synapsis completely fill it (fig. 76). The spireme is made up of a number of threads which seem to be composed of single granules, though the linin threads are double, and in a few cases (smear preparations) there was some evidence in these granules of a longitudinal split. At this stage (fig. 75) a large deeply staining body appears for the first time and becomes more pronounced as the basi-chromatin loses its staining reactions, and throughout the growth stage of the spermatocyte this body retains its staining reaction and may be seen either as a single or dumbbell-shaped structure. The chromatin now resumes its staining property and the chromosomes appear as broken segments of the spireme usually bent or twisted at their center; then these

segments begin to condense and a longitudinal split is seen in them (fig. 81). Though the number of segments at this time could not be counted, yet it was plainly the reduced number, and this reduction must have taken place during the last few stages when, because of the deeper staining and greater massing of the chromatin, it was impossible to follow the steps by which this pseudo-reduction occurred. The ring chromosomes of the prophase are seen to be formed by the coming together of the split ends and the gradual condensation of the chromatin mass: this concentration is complete in sections, and dumbbell-shaped chromosomes and occasionally tetrads are formed, which are connected by linin threads (figs. 83–85). At this stage of prophase, when growth has reached its greatest extent, the cell is several times as large as in the beginning of the growth period, and the nucleus, which has increased in size with the cell, is usually eccentrically placed. At this time chromatin granules similar to those described for *C. promethea* are found in the cell cytoplasm and are traceable throughout all succeeding steps.

A metaphase of the first maturation division shows thirty chromosomes (fig. 87) which are symmetrically placed; their division is equal and reducing. The chromosomes of the first polar plate are too cendensed to count and quickly pass through the prophase of the second maturation. The centrosomes move so that the second division is at right angles to the first and the chromosomes quickly arrange themselves for the second division, in which, as in the first, there is an equal division of the chromatin: an equatorial plate of the second spindle shows thirty chromosomes, smaller than in the first and always single (fig. 92). The distinct zwischenkörper and a number of the chromatin granules mentioned above are seen during telophase both in the cytoplasm and upon the mantle fibres (Pl. XXV, fig. 98). The development of the spermatid shows no new features, but corresponds very closely to that described for *C. promethea.*

Centrosomes are seen throughout all stages: in the resting spermatocyte they appear as small paired structures closely pressed to the cell membrane and with the short flagellæ extending into the lumen (fig. 79); as development proceeds these centrosomes move nearer to the nucleus and migrate to opposite sides of the cell in preparation for the first division, and in late prophase and anaphase stages they appear as dumbbells and are so conspicuous by their size and prominence that they might almost be taken for very small chromosomes. Astral rays are well developed and, so far as could be determined, these grow out directly from the centrosomes.

Automeris io.

This species was found abundantly about Lansdowne, Pa., feeding upon maple trees and rose bushes. The larvæ pupated in September and showed comparatively rapid development. The paired testes of *Io* resemble those of other members of the Saturnids in position and color, but differ in shape; instead of the kidney-shaped body, such as has been described for *C. promethea* and *I. polyphemus*, each of the four lobes of which it is composed is rounded and distinct, and the testes become elongate by the two lateral lobes meeting in the centre and the two distal ones being pushed longitudinally. As in the other forms each testis has but a single vas deferens.

Secondary spermatogonia are comparatively large cells with few scattered chromatin granules and a rather large plasmosome which stains like chromatin and shows a loose structure (figs. 98 and 99), and from which, as a center, short chromatin threads are seen to radiate, suggesting the karyosphere described by Blackman (1905 b) for *Scolopendra*. Figs. 100, 101, and 102 show three stages in the last spermatogonial divisions: controsomes are present with aster and spindle fibres well developed, division is equal, and in telophase the massing of the chromosomes is complete. The spermatogonial plates were so condensed that all attempts to determine the number of the chromosomes were unsuccessful.

In going into the contraction stage the chromatin becomes looped on the side of the nucleus, which lies toward the greater amount of cytoplasm (figs. 103 and 104); then the loops begin to loosen, and in early post-synapsis is seen for the first time a round darkly staining body (fig. 106) which at this stage is quite small, but, as the skein gradually loosens and the loops extend more and more into the nucleus, this body, the idiochromosome, becomes more marked, and during the growth period it is seen either as one or two round dark-staining bodies. During this stage the centrosomes lie very near the inner cell wall and from them flagellæ grow out into the lumen of the cyst. Fig. 109 shows a cell in the early rest stage; here the centrosomes are single and the flagellum which grows from each is quite short. Fig. 110 shows a similar stage where the cell has grown to about twice the size of that in fig. 109; in this, the centrosomes have divided and the flagellum which grows from each centrosome is relatively long.

In preparation for prophase it may be seen that the spireme has broken into segments which are of various shapes and, in most cases, so bent at the center as to suggest their formation by the union of two chromosomes (fig. 111). These now split and join end to end to

form the bipartite and quadripartite chromosomes of the prophase (figs. 114, 115 and 117), which in *io* show a far greater variety of form than in any of the other species described. In the very beginning of the prophase, when the chromosomes are forming from the split section of the spireme, the idiochromosome so compact and darkly staining throughout the growth period, may be seen to be composed of two parts, a paler-staining plasmosome and beside and upon this a chromatin mass: later the plasmosome fades and this peculiarly formed chromosome is no longer distinguishable from the others. A spermatocyte equatorial plate shows thirty-one chromosomes, and the metaphase and anaphase of the first maturation shows that this division is equal and reducing (figs. 119 and 120). The second division is also equal and separates whole chromosomes along the longitudinal axis.

Throughout almost every step the centrosome can be traced as a dark granule surrounded by an archoplasmic mass. As prophase advances, astral rays are seen about the centrosomes, which at this time have divided and appear as dumbbells, and from them astral fibres radiate in all directions, becoming especially well developed on the side toward the nucleus. Of equally marked development are the spindle fibres, which in the telophase of the second division may be traced directly to the nebenkern of the spermatid, while a clear vesicle, the idiozome, lying on one side of the nucleus of the spermatid, can also be traced to the archoplasmic mass surrounding the centrosome of former stages.

The cells, which after the second division are irregularly placed, are now arranged with their nuclei against the wall of the cyst so that the entire wall is covered with the heads of the spermatozoa, while the tails project into the lumen (Pl. XXVI, figs. 131 and 132). During the development of the spermatozoon marked changes occur in the nucleus: the chromatin becomes granular and scattered and the nucleus decreases in size and frequently bends through an angle of 90° to 180°; later, when the condensation of the nucleus becomes more marked and the tail elongates, the cells again change their position, coming to lie with their heads crowded together and their tails parallel (figs. 133, 134 and 135).

Samia cecropia.

This species was found abundantly on maples and many bushes about Philadelphia. Development is very similar to that of the other forms described. The resting spermatogonia show one or two net knots; these are more compact than those of other species and, unlike them, are surrounded by a clear area. Figs. 140–142 show

stages of the last spermatogonial division; here the chromosomes in metaphase are too crowded to count. A thin skein is formed preparatory to synapsis (fig. 143) and by condensation of this the chromatin becomes collected at one side of the nucleus (fig. 144); later it spreads through the nucleus and gradually fills it (figs. 145 and 146). In the growth stage which follows we find the same large, deeply-staining body described for the other species. The centrosomes which have been followed since the last division of the spermatogonia are seen at this time as two small dark bodies near the nuclear membrane (fig. 147); these may be clearly seen to pass through the regular cycle of changes, a cycle which is repeated in each maturation division, and which in many respects may be parallel with the changes which take place during division of the nucleus. In *S. cecropia,* as in all the other species here studied, the centrosomes seem to be continuous from generation to generation as is the nucleus itself. The chromosomes, in smear perparations, retain their outlines during the growth stage (fig. 148); in this the nucleus has been separated from the cytoplasm and much stretched in drying. Corresponding almost exactly with similar stages in *C. promethea,* we have the gradual condensation into rings and dumbbells, while the idiochromosome, which retains its continuity longest, finally assumes the same form as the others and is indistinguishable from them. Division in first and second maturations closely follows that of the other Saturnids, and the remnants of the spindle fibers which surround the chromosomes of the last division and extend far into the cell cytoplasm may be seen to go directly into the spermatid and there form the nebenkern. The spermatozoon has here been traced to its complete development. The earlier stages correspond to those described for other members of the family, but as development proceeds (Pl. XXVII, figs. 167–170), the decrease in size of the nucleus and elongation of the tail seem to be connected with the extension of the head piece, which at this time appears as a cytoplasmic projection containing a darker body and only differing from the rest of the cytoplasm in its greater clearness. While the chromatin has been condensed into a small round mass which completely fills the nucleus, the head piece has increased in length and has become pointed and shield shaped, and the axial filament has grown very long. The final stage of development shows a pointed head piece with its acroblast, a long narrow nucleus, a very slightly developed middle piece, and an exceedingly long tail which, due to a twisting of the inner and outer membranes about the axial filament, has the appearance of a spiral.

Acronycta sp.

A small cocoon found upon a *Cecropia* cocoon was identified by Prof. John B. Smith of Rutger's College as an *Acronycta*, possibly *oblinita*. By comparison with *oblinita* it was found that this was not the species, but because of certain unusual structures in the cytoplasm of both spermatocytes and spermatids it seemed of sufficient interest to be included in the present study. This material was fixed with the same care and by the same methods as were used for other forms so that the structures here described cannot be the result of bad preservation.

The testes which in all the Saturnids remain paired are fused in this form along the midline, suggesting a similar condition mentioned for butterflies. In the rest stage of the first spermatocyte the nucleus shows the usual deeply-staining nucleolus and a fine linin meshwork, while in the cell cytoplasm beside the centrosomes a darkly-stained body appears (figs. 171 and 172); except for its greater size, this body appears and behaves like the "chromatin granules" described for the family of Saturnids, and like them may be seen to divide and migrate to opposite poles of the cell (figs. 178 and 179). In addition to this an irregular mass is often found which in form and staining reaction resembles chromatin and looks as though it had been thrown out of the nucleus; though this suggests the Mitochondria of Benda (1899) and similar bodies described by Meves (1902) and Schreiner (1906) and is traceable in many cells throughout all stages, yet it was not seen to take any part in cell development. In the cytoplasm in all stages of prophase (figs. 173 and 174) both "chromatin granule" and Mitochondria are to be seen; the former as divided and beginning to migrate; the latter as an inert mass. In the first maturation division (fig. 177) a partial division of the Mitochondria is seen, while figs. 176, 178 and 179 show various stages in the equal division of the chromosomes and of the "chromatin granule." One thing which is very noticeable here in contrast to all other forms is the position of the spindles which lie to one side of the cell, while the chromatin granule and Mitochondria occupy the other side; an exception to this is seen in fig. 176, where one of the "chromatin granules" is placed upon the spindle. A metaphase of the second maturation division shows, in addition to a chromatin granule and a Mitochondria twenty-nine chromosomes. By an apparently equal division of the chromatin granules each spermatid receives not only a nucleus and a nebenkern from the mother cell, but also a "chromatin granule" surrounded by a clear zone; this granule at first lies beside the nucleus of the spermatid,

but soon moves toward the head, where it takes its final position and appears to be transformed into the acrosome.

Spermatids and Spermatozoa.

The metamorphosis of the spermatid of each species here studied has in most of its parts been considered in the preceding text; thus, as has been pointed out, the sperm head is made up of the modified nucleus of the second spermatocyte plus the head shield, while what in many animals would correspond to the middle piece is in Lepidoptera only the region occupied by the two centrosomes. The nebenkern which seems to be common to all insects refers, when used in its original sense (Bütschli, 1871), to that body in the spermatid which is formed at least in part by the spindle fibres of the second maturation division and which later gives rise to the inner tail membrane. Many workers on insects, among whom may be mentioned La Valette St. George, Platner, Erlanger, Henking, Wilcox (1896), and Paulmier, trace its origin at least in part to the remnants of the spindle, though Paulmier attributed only a small part to the spindle fibres, believing that the nebenkern in *Anasa* is formed largely from the yolk mass. Meves (1901), on the other hand, claims that this body is built up independently of the spindle fibres out of granules which were present in earlier generations, and which are identical with the yolk granules of Paulmier, the Cytomitosomen of La Valette St. George, and the Mitochondria of Benda. No trace of yolk granules were found in the Saturnids and the dark-staining accessory masses figured for *Acronycta* were found to take no part in the formation of the nebenkern; but, in both smear preparations and sections, the nebenkern material for all the species studied was clearly traceable to the spindle fibres of the second spermatocytic division.

The problematical parts which remain to be discussed are the subsequent history of the chromatin granule and the origin of the head piece and axial filament. Many writers describe the head piece or acrosome as arising from the idiozome which migrates to the anterior pole of the cell. The Schreiners (1908) have shown that the head piece of Myxine is made up of two separate Anlagen, the primary and secondary head vesicles, which are only joined in the beginning of sperm ripening: the primary head vesicle formed from the sphere takes its place after the second division, while the secondary head vesicle remains in the opposite part of the cell near the centrosome and only reaches its final position during the beginning of sperm ripening. A third view is that of Lenhossék (1899), who derives the

acrosome from the cytoplasm. The acrosome in Lepidoptera is formed from two parts—a clearer vesicle and a dark granule, but the idiozome which migrates from the acrosomal region in early development has not been seen to return, nor has it been possible to trace the sphere material to this region; it therefore seems most probable that the acrosome in Lepidoptera has been formed from modified cytoplasm. The chromatin granule noted throughout preceding pages and described as a small granule staining in iron hæmatoxylin and surrounded by a clear zone, is seen in very young spermatids to lie quite near the nucleus and in a position corresponding to the idiozome; in *Acronycta* this granule moves nearer the head of the nucleus, and a similar granule is later found within the head piece; the same relation is suggested in the Saturnids. The presence of this body recalls the "chromatin body" described by Lenhossék (1898) as an extruded nucleus of unknown origin which degenerates without taking any part in the formation of the spermatozoon, as well as a similar body shown by the Schreiners (1908) to be present in *Myxine*. King (1907) finds such a body in Amphibia, traces it from the primary spermatogonia to the acrosome of the spermatozoon, and because of its function names it "acroblast." The extrusion of the chromatin granules from the nucleus during the growth stage like that described for Myxine has here been observed, but although it persists and is traceable through the spermatid, yet further research is required to determine whether this chromatin granule of Lepidoptera is really functional or whether like similar granules described by other workers it plays no part in the development of the sperm.

The origin of the axial filament from one of the centrosomes of the middle piece was first demonstrated by Moore (1895) for Elasmobranchs. Paulmier described a similar origin for *Anasa* and gave proof for this by the discovery of the occurrence in giant spermatids of two and four axial filaments. That the axial filament actually arises from the centrosomal substance, or is formed like astral rays and spindle fibres by a differentiation of the cytoplasm, are the two views most widely held. Meves (1897) in his work on *Pygæra* accepts the latter view, believing that "die Fäden der Schmetterlingspermatocyten extracellular gewordene Mitomfäden darstellen." The same writer concludes for *Lithobius* "dass die bei Lithobius beobachteten Fäden sind aus dem einen der beiden Centralkörper durch Längenwachstum desselben, hervorgegangen," and Korff (1899) on *Helix* and Suzuki (1898) on Elasmobranchs show that the inner centrosome elongates and so support the first view of Meves that this

contractile element may be derived directly from the centrosomal substance. The precocious attempt of the centrosome in the first and second spermatocytes to form a flagellum as has been described by Meves, Henneguy, and in the present work for three species of Saturnids, and *D. archippus* (fig. 191) and *P. cresphontes* (fig. 192) lends weight to the view that at least in Lepidoptera the out-growth of the axial filament from the centrosome is comparable to the formation of spindle fibres and astral rays. This suggests a possible relationship with other ciliate cells of both plants and animals, and in comparing the vibratile cilia of Lepidoptera with those of plants one is struck with the analogies which exist: the dark-staining granule at the base of the cilium is similar both in appearance and position to the centrosome of the spermatocytes, while the axial filaments in both cases are similar in appearance, vibratile nature, and the part they play in fecundation. Such centrosomes have been figured and described by Webber (1897) for *Zamia* and by Ikeno (1894) for *Cycas* and *Ginkgo*, though the two authors differ in their interpretations; Webber believing that these are only "centrosome-like," while Ikeno does not hesitate to consider them as true centrosomes similar to those of animal spermatozoa. Ishikawa's (1899) observations that the flagellum of *Noctiluca* grows out from the centrosomal end of the cell, its substance apparently arising from the central spindle, strengthens this view, while Belajeff's (1897) comparison of the spermatogenesis of Characeæ, Filicineæ, and Equisetaceæ with such animal forms as the Salamander shows a close relationship between the cilia of spermatozoids and the axial filament of spermatozoa.

IV. Theoretical Considerations.

As long ago as 1885 Rabl concluded that each chromosome is a persisting individual and not a structure formed anew in each generation. Van Beneden (1883) advanced the theory of individuality of the chromosomes by pointing out that there is a constant number of chromosomes for each species, always half this number in each maturation division and that the number is restored by fertilization. This does not mean that the chromosomes remain unchanged, but that a chromosome of any generation is the descendant of a particular chromosome of a preceding generation. This view was strongly supported by Sutton (1903) who, basing his theory on his own cytological work as well as upon Boveri's (1902) experimental work on the sea urchin egg, concluded that the chromosomes must be the seat of particular qualities, and showed, as Montgomery (1901) had done,

that synapsis is brought about by the conjugation of maternal and paternal chromosomes of the same size. This led to the view that homologous chromosomes represent homologous characters and only by the definite association of chromosomes of certain characters can Sutton's theory of the purity of the germ cells be maintained.

Individuality is further extended by establishing a definite number of chromosomes for each species. The number of chromosomes found in the equatorial plates of the family Saturnids ranges from thirteen in *P. cynthia* to thirty-one in *A. io*, and there is no evidence, that *P. cynthia* is more highly developed than other members of the family. This is in accord with the view of Montgomery (1906), who after tabulating the number of chromosomes in several hundred species of plants and animals, was forced to give up his theory of a correlation between the number of chromosomes and the evolutionary stage of the species. McClung (1905) claims that the family Acrididæ is characterized by a fixed number of chromosomes which is constant for all the genera and species: the genera are characterized by a definite arrangement and association, while the species show the same grouping as the genus but are distinguished by the size difference of the chromosomes and spindles. He considers that a definite series of chromosomes accompanies a group of somatic characters used by systematists for classification. That the chromosomes may be of classificatory significance has already been mentioned for *cynthia* and *promethea*, where the external difference of the pupa was often so slight that only by reference to the number of chromosomes could the species be definitely determined; this was, however, by the marked difference in number, not in arrangement.

If this individuality is to be maintained, maturation mitoses must show one transverse and one longitudinal division, and while the end result is the same, whether the first division is transverse or longitudinal, yet it seems probable, as Montgomery (1903) points out, that the first division will be found in all cases of heterotypic division to be reducing. By a comparison of the forms here described with *P. cynthia*, where twenty-six pairs of granules can be counted in the spermatogonia and thirteen in the first spermatocyte, I conclude that pseudo-reduction takes place in the family Saturnidæ either during the telophase of the last spermatogonic division or during synizesis by an end-to-end union of homologous chromosomes, and that the angle in the middle of the rods (fig. 22) and the constriction in the rings (fig. 26) and dumbbells marks this point of union of univalent chromosomes, as well as the point of separation of the first division.

The longitudinal split of the post-synaptic stages which corresponds to a similar split in the spermatogonia and remains open and traceable until the chromosomes are upon the spindle might be assumed to be a side-by-side union considered by A. and K. Schreiner (1904) to be "the" method of conjugation, and as such I at first interpreted it; but by a careful study of the steps in the formation of the chromosomes from the segmentation of the spireme to the prophase, viz., the opening of the chromatin segments into rings and their linin connection, it becomes clear that the Weismannian method of reduction could only be brought about if conjugation had taken place by an end-to-end union, as first interpreted by Montgomery (1900), for only in this way would there be one transverse and one longitudinal division, so separating univalent chromosomes. A conjugation by parasynapsis would result in two reduction divisions and the individuality of the chromosomes would be destroyed. I am led to conclude that this longitudinal split is a precocious division early laid down to mark the plane of the second maturation division.

The accessory chromosome first described by Henking (1891) for *Pyrrochoris apterus* and later by McClung (1899), Sutton (1902), and Baumgartner (1904) for Orthoptera, Blackman for Myriopoda, Wallace (1900) for Arachnida, Paulmier (1899), Montgomery (1898, 1901, 1906), Wilson, Stevens, etc., for Hemiptera, Stevens and Nowlin (1906) for Coleoptera, and Lefevere and McGill (1908) for Odonata, has by its peculiar behavior gone far toward establishing the theory of individuality of the chromosomes. McClung was the first to suggest that the accessory chromosome might be a sex determinant, believing that this chromosome was peculiar to the sperm; but Stevens and Wilson, while corroborating this suggestion of sex determination, showed by a comparison of the equatorial plate of somatic cells and germ cells of both sexes that it is the female and not the male that possesses this additional chromosome.

In his arrangement of the Heteroptera into three groups according to the three types of spermatozoa, Wilson has brought all cases into harmony with the dimorphism theory and has given direct evidence of the conjugation of maternal and paternal chromosomes. The first class is one in which there is a single heterotropic chromosome resulting in two classes of spermatozoa of which one-half possesses, one-half lacks this element; in the second class the male has the same number of chromosomes as the female but possesses one large and one small idiochromosome while the female possesses two large chromosomes; and in the third class the idiochromosomes are equal in size in both

sexes, though because of certain peculiarities of behavior the equal pair of idiochromosomes may be considered as representing different characters, so that the dimorphism, though masked, may nevertheless be considered present in this class also.

The question arises, can the species of Lepidoptera here studied be brought into relation with the theory of dimorphism and individuality of the chromosomes?

A careful examination of the chromatin element in the species here described shows, as has already been pointed out, that one chromatin element acts differently from the others during a certain period in the development of the germ cells. It is distinguished from all other cell structures by its staining reaction, its precocious division, and its close association with a plasmosome; while later it shows likeness to the other chromosomes in form, valence and division; such behavior makes it necessary to interpret this, as other workers have done, as an equal pair of idiochromosomes representing different characters from those of the other chromosomes and expressing by their peculiar behavior a masked dimorphism.

The present study of Lepidoptera offers no such support to the theory of the individuality of the chromosomes as Sutton found in *Brachystola* and other workers have found in Hemiptera, yet the following facts are evidence in favor of this theory; (*a*) that the number of chromosomes remain the same from generation to generation, (*b*) that they are seen in maturation divisions to be formed of pairs of equal size, (*c*) that in smear preparations the boundaries can be traced and the chromosomes never entirely lose their continuity during the growth stage, and (*d*) that at least one of the chromatin elements shows marked peculiarity in its behavior, and can therefore be traced throughout the growth period. These facts show that Lepidoptera like the other insect orders may be brought into harmony with recent cytological work.

V. Summary.

1. The spermatogonia contain an equal number of chromosomes of about the same size and shape, and in the family of Saturnids a net knot of chromatin granules is always found during the resting stage.

2. During the growth period of the spermatocyte a dense body is found which is either single or dumbbell shape, is eccentrically placed, and stains like basi-chromatin; in *T. polyphemus* and *A. io* it first appears in the early post-synapsis stage, though in other species it is

not distinguishable until the chromatin loses its staining properties. Later this body shows a clearer plasmosome part with the chromatin in a band or in scattered granules, and from this chromatin a chromosome is formed which has the same valence as the other chromosomes and is indistinguishable from them. Because of its behavior this structure is comparable to similar bodies described by Wilson and may be considered as an equal pair of idiochromosomes.

3. The spireme segments into the reduced number of chromosomes and by condensation the rings become the dyads and tetrads of the first maturation division. By the Foot-Strobell (1907) method of smear preparations, the longitudinal split could be seen in both post-synapsis spiremes and early prophase and its relation traced to tetrad formation and succeeding maturations. The first maturation division is reducing separating univalent chromosomes, while the second is longitudinal and equational.

4. The same number of chromosomes is found in the equatorial plate of both first and second spermatocytes and by equal divisions the spermatids each receive similar chromosome groups, so that there is no visible dimorphism of the spermatozoa.

5. Centrosomes were traced from the secondary spermatogonia throughout the whole development of the germ cells. Aster rays and spindle fibres are well developed and the difference between these is especially well shown in mitotic figures in *A. io.*

6. A chromatin granule enclosed by a clearer area is first found in the cytoplasm of the growth stage. This has been described for all forms and in addition an accessory chromatin-like mass has been figured for *Acronycta;* whether the chromatin granule really functions as the acroblast of King cannot be determined without further research. The accessory mass is present in only part of the cells and is seen to degenerate.

7. A precocious attempt of the centrosomes to form a flagellum is seen in three of the species of moths studied and in two butterflies. In *Promethea* this flagellum has been traced from the early growth stage of the first spermatocyte through prophase, second spermatocyte, and early spermatid into developed spermatozoa, while it has also been described and figured for the growth stage of *A. io, T. polyphemus, P. cresphontes,* and *D. archippus.* This adds additional weight to the view that the axial filament grows out from the centrosome and suggests that its origin is similar to that of astral rays and spindle fibres.

VI. Literature.

Baumgartner, W. J. 1904. Some New Evidence for the Individuality of the Chromosomes. Biol. Bull., VIII.

Belajeff, W. 1897. Ueber die Aehnlichkeit einiger Erscheinungen in der Spermatogenese bei Thieren und Pflanzen. Ber. Deutschen Bot. Ges., XV, 342.

Benda, C. 1899. Weitere Mittheilungen über die Mitochondria. Verh. d. phys. Ges. zu Berlin.

Van Beneden, E. 1883. Recherches sur la maturation de l'oeuf, la fécondation et la division cellulaire. Arch. de Biol., 4.

Blackman, M. W. 1905. (*a*) Spermatogenesis of Myriopods: III. Spermatogenesis of Scolopendra heros. Bull. Mus. Comp. Zool. Harvard, XLVIII.

1905. (*b*) Spermatogenesis of Myriopods: IV. On the Karyosphere and Nucleolus in the Spermatocyte of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Science, XLI.

Boring, A. M. 1907. A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ. Jour. Exper. Zool., Vol. IV, No. 4.

[1] Boveri, T. 1902. Ueber Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh. d. Phy. Med. Ges. zu Würzburg, N. F., Bd. XXXV.

Buetschli, O. 1871. Vorläufige Mitteilung über Bau und Entwickelung der Samenfäden bei Insecten und Crustaceen. Zeitsch. f. wiss. Zool., Bd. 21, Vol. 4.

Carnoy, J. B. 1884. Cytodiérèse des Arthropodes. La Cellule, I.

Cholodkovsky. 1894. Zur Frage über die Anfangstädien der Spermatogenese bei den Insekten. Zool. Anz., V, 17.

Conklin, E. G. 1902. Karyokinesis and Cytokinesis. Jour. Acad. Nat. Sci. Phila., Sec. Sev., XII.

Dederer, P. H. 1907. Spermatogenesis in Philosamia cynthia. Biol. Bull XIII, 2.

Erlanger, 1896. Ueber den sogenannten Nebenkern in den männlichen Geschlechtszellen der Insekten. Zool. Anz., XIX.

Foot, K. and E. C. Strobell, 1905. Prophase and Metaphase of the First Maturation Spindle of Allolobophora fœtida. Amer. Jour. Anat., IV.

Henking, H. 1891. Ueber Spermatogenese und deren Beziehung zur Entwickelung bei Pyrrhocoris apterus. Zeit. wiss. Zool., 51.

Henneguy, L. F. 1898. Sur les rapports des cils vibratiees avec les centrosome. Arch. Anat. Mik., I.

Ikeno, S. 1898. Untersuchungen über die Entwickelung der Geschechtsorgane, etc., bei Cycas. Jahr. wiss. Bot., XXXII, 4.

Ishikawa, M. 1899. Further Observations on the Nuclear Division of Noctiluca. Jour. Coll. Sci. Tokyo, X, 2.

King, H. D. 1907. The Spermatogenesis of Bufo lentigenosus. Amer. Jour. Anat., VII, 3.

Korff, 1899. Zur Histogenese der Spermien von Helix. Arch. m. Anat., LIV.

Lefevre, G., and McGill, C. 1908. The Chromosomes of Anasa tristis and Anax junius.

v. Lenhossek, M. 1898. Untersuchungen über Spermatogenesis. Arch. m. Anat., LI.

McClung, C. E. 1899. A Peculiar Nuclear Element in Male Reproductive Cells of Insects. Zool. Bull., II.

1900. Comparative Study of Hippiscus with Other Orthoptera. Sci. Bull. Kans. Univ., IX.

1901. Notes on Accessory Chromosomes. Anat. Anz., XX.

1902. (*a*) Spermatocyte Development of Locustidæ—Kans. Univ. Bull., IX.

[1] I did not have access to this paper.

1902. (*b*) The Accessory Chromosome. Sex Determinant(?) Biol. Bull., III.
1905. Chromosome Complexes of Orthoptera Spermatocytes. Biol. Bull., IX, 5.

MEVES, F. 1897. Ueber Centralkörper in männlichen Geschlechtszellen von Schmetterlingen (Pieris brassicæ, Mamestra brassicæ, Pygæra bucephala, Sphinx euphorbiæ, Sphinx ligustri, Harpyia vinula). Anat. Anz., XIV, 1.

MEVES, F. 1901. Struktur und Histogenese der Spermien. Ergebnisse Anatomie, II.

MONTGOMERY, T. H. 1898. Spermatogenesis of Pentatoma. Zool. Jahrb., XII.
1900. Spermatogenesis of Peripatus. Zool. Jahrb., XIV.
1901. A Study of the Chromosomes of the Germ Cells of Metazoa. Trans. Amer. Phil. Soc., 20.
1903. The Heterotypic Maturation Mitosis in Amphibia and its General Significance. Biol. Bull., 1903, Vol. 4.
1904. Some Observations and Considerations upon the Maturation Phenomena of the Germ Cells. Biol. Bull., 6.
1906. Chromosomes in the Spermatogenesis of the Hemiptera Heteroptera. Trans. Amer. Phil. Soc., N. S., XXI.

MOORE, J. E. S. 1895. On the Structural Changes in the Reproductive Cells during Spermatogenesis of Elasmobranchs. Quart. Jour., XXXVIII.

MUNSON, J. P. 1906. Spermatogenesis of the Butterfly, Papilio rutulus. Bos. Soc. Nat. Hist., 33.

NOWLIN, W. N. 1906. A Study of the Spermatogenesis of Coptocycla aurichalcea and Coptocycla guttata, with special reference to the Problems of Sex Determination. Jour. Exp. Zool., III.

PAULMIER, 1899. Spermatogenesis of Anasa Tristis. Jour. Morph., XV, Supplement.

PLATNER, G. 1886. Die Karyokinese bei den Lepidoptera als Grundlage für eine Theorie der Zelltheilung. Internat. Monatsch. f. Anat. Hist., III.

RABL, C. 1885. Ueber Zelltheilung. Morph. Jahrb., X, 1885.

VOM RATH, O. 1892. Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris. Arch. mik. Anat., XL.

SCHREINER, A. and K. E. 1904. Die Reifungsteilungen bei den Wirbeltieren. Anat. Anz., XXIV.
1906. Neue Studien über die Chromatinreifung der Geschlechtszellen. Archiv. de Biol., 22.

STEVENS, N. M. 1905. Studies in Spermatogenesis: I–II. A Comparative Study of the Heterochromosomes in Certain Species of Coleoptera, Hemiptera and Lepidoptera, with especial reference to Sex Determination. Carnegie Inst. Publicat., No. 36, II.

SUTTON, W. S. 1900. Spermatogonal Divisions in Brachystola magna. Kans. Univ. Quart., IX.
1902. Morphology of the Chromosome Group in Brachystola magna. Biol. Bull., IV.
1903. The Chromosomes in Heredity. Biol. Bull., IV.

SUZUKI, B. 1898. Notiz über die Entstehung des Mittelstückes von Selachiern. Anat. Anz., XV, 8.

TOYAMA, 93. Preliminary note on Spermatogenesis of Bombyx Mori. Zool. Anz., No. 438.

V. LA VALETTE ST. GEORGE. 1887. Spermatologische Beiträge. Arch. f. Mikr. Anat., XXX.
1897. Zur Samen und Eibildung bei Seidenspinnen (Bombyx mori). Arch. für Mikr. Anat., 50, p. 751.

VERSON, E. 1889. Zur Spermatogenese. Zool. Anz., XII.
1894. Zur Spermatogenese bei der Seidenraupe. Zeit. wiss. Zool., 58.

WALLACE, L. B. 1900. The Accessory Chromosomes in the Spider. Anat. Anz., 18.

WEBBER, H. J. 1897. Peculiar Structures occurring in the Pollen-tube of Zamia. Bot. Gazette, XXXIII, 6 and XXXIV, 1.

WEISMANN. Essays on Heredity, II.

WILCOX, E. V. 1896. Further Studies on the Spermatogenesis of Caloptenus femurrubrum. Bull. Mus. Comp. Zool. Harvard, XXIX.

WILSON, E. B. 1902. The Cell in Development and Inheritance.
1905. (*a*) Studies on Chromosomes: I. The Behavior of the Idiochromosomes in Hemiptera. Jour. Exp. Zool., II.
1905. (*b*) Studies on Chromosomes: II. The Paired Microchromosomes, Idiochromosomes and Heterotypic Chromosomes in Hemiptera. Jour. Exper. Zool., II.
1906. Studies on Chromosomes: III. Sexual Difference of Chromosomes Group in Hemiptera, with some Consideration on Determination and Inheritance of Sex. Jour. Exper. Zool., III.

DESCRIPTION OF PLATES XXII–XXVII.

All the figures, with the exception of the text figure, page 10, were drawn at the level of the table with the aid of a camera lucida. A Zeiss microscope was used with a No. 8 ocular and a 16 mm. oil immersion objective, giving a magnification of 2,250 diameters. The plates have been reduced to about one-third their original size.

PLATE XXII—*Callosamia promethea*—Figs. 1–4—Spermatogonial rest stages, showing stages in the disappearance of the net knot.
Fig. 5—Spermatogonial spireme.
Fig. 6—Spermatogonial equatorial plate showing 38 chromosomes.
Fig. 7—Spermatogonial metaphase.
Fig. 8—Spermatogonial anaphase.
Figs. 9 and 10—Spermatogonial telophase showing formation of nuclear membrane.
Fig. 11—Formation of spireme preparatory to synizesis. Spermatocyte.
Fig. 12—First spermatocyte, synizesis.
Fig. 13—First spermatocyte, synizesis (smear preparation).
Fig. 14—First spermatocyte, chromatin in loops.
Figs. 15–18—Degenerating cells.
Fig. 19—First spermatocyte, early post, synapsis (smear preparation).
Figs. 20–21—First spermatocyte, late post-synapsis (smear preparation).
Fig. 22—First spermatocyte, spireme divided into reduced number of chromosomes.
Figs. 23–26—First spermatocyte, growth stage.
Fig. 26—First spermatocyte, smear preparation showing continuity of the chromosomes.
Figs. 24, 25 and 29—First spermatocyte, ciliated centrosomes.
Fig. 27—First spermatocyte, split segments.
Figs. 32, 33—First spermatocyte, ring formation.
Fig. 28—First spermatocyte, condensation of rings.
Fig. 34—First spermatocyte, formation of plasmosome.
Figs. 35–38—First spermatocyte, stages in prophase of first maturation division.
Fig. 39—First spermatocyte, smear preparation, late prophase showing plasmosome with chromatin band.

PLATE XXIII—*Callosamia promethea*—Fig. 40—First spermatocyte, equatorial plate, 19 chromosomes.
Fig. 41—First spermatocyte, polar plate, 19 chromsomes.
Fig. 42—First spermatocyte, metaphase, lateral view.
Fig. 43—First spermatocyte, anaphase.
Fig. 44—First spermatocyte, telophase.
Fig. 45—Second spermatocyte, polar view.
Fig. 46—Second spermatocyte, prophase, chromatin scattered, chromatin granule present.
Fig. 47—Second spermatocyte, prophase, rearrangement for second division, zwischenkörper marks cell axis.
Fig. 48—Second spermatocyte, prophase, two chromatin granules present, ciliated centrosomes.

Fig. 49—Second spermatocyte, Equatorial plate, 19 chromosomes.
Fig. 50—Second spermatocyte, metaphase spindle.
Figs. 51 and 52—Second spermatocyte, anaphase spindle.
Fig. 53—Second spermatocyte, telophase.
Fig. 54—Spermatid, first stage, chromatin granule present.
Fig. 55—Spermatid, degenerating.
Fig. 56—Spermatid, first stage showing chromatin granule, ciliated centrosome and chromatin nucleolus.
Figs. 57 and 58—Spermatid, second stage.
Fig. 59—Spermatid, formation of axial filament.
Figs. 60 and 61—Spermatid, later stages.

PLATE XXIV—*Telea polyphemus*—Fig. 62—Early spermatogonial stage, showing net knot.
Fig. 63—Late spermatogonial stage.
Figs. 64 and 65—Spermatogonial spireme.
Fig. 66—Spermatogonial, equatorial plate, probably 60 chromosomes.
Figs. 67 and 68—Last spermatogonial, division.
Fig. 69—Spermatogonial telophase (oblique section).
Fig. 70—First spermatocyte, spireme preparatory to synizesis.
Fig. 71—First spermatocyte, beginning of synizesis.
Fig. 72—First spermatocyte, synizesis of smear preparation.
Fig. 73—First spermatocyte, synizesis.
Fig. 74—First spermatocyte, coming out of synizesis.
Fig. 75—First spermatocyte, early post-synapsis, showing plasmosome.
Fig. 76—First spermatocyte, late post-synapsis.
Fig. 77—First spermatocyte, fading of spireme.
Fig. 78—First spermatocyte, growth stages.
Fig. 79—First spermatocyte, ciliated centrosomes.
Fig. 80—First spermatocyte, segmented spireme, showing plasmosomes.
Fig. 81—First spermatocyte, segmented spireme, showing split.
Figs. 82–85—First spermatocyte, showing various stages in prophase.
Fig. 86—First spermatocyte, early metaphase.
Fig. 87—First spermatocyte, equatorial plate, 30 chromosomes.
Fig. 88—First spermatocyte, metaphase spindle, showing "chromosome granule."
Fig. 89—First spermatocyte, anaphase.
Figs. 90 and 91—Second spermatocyte, rearrangement of chromosome for second division.
Fig. 92—Second spermatocyte, equatorial plate, 30 chromosomes, "chromatin granules."
Fig. 93—Second spermatocyte, anaphase.
Fig. 98—Second spermatocyte, telophase, showing zwischenkörper and chromatin granules.
Fig. 94—Spermatid, early stage.
Fig. 95—Spermatid, second stage.
Figs. 96 and 97—Spermatid, later stage, formation of axial filament.
Fig. 191—Ciliated centrosome (*Danais archippus*).
Fig. 192—Ciliated centrosome (*Papilio cresphontes*).

PLATE XXV—*Automeris io*—Fig. 98*a*—Early spermatogonial rest stage.
Fig. 99—Later spermatogonial rest stage, showing curious net-knot.
Fig. 100—Spermatogonial metaphase.
Fig. 101—Spermatogonial anaphase.
Fig. 102—Spermatogonial telophase.
Fig. 103—First spermatocyte, going into synizesis.
Fig. 104—First spermatocyte, later stage.
Fig. 105—First spermatocyte, complete condensation.
Figs. 106 and 107—First spermatocyte, early post synapsis.
Fig. 108—First spermatocyte, later post-synapsis showing plasmosome.
Fig. 109—First spermatocyte, growth stage, double plasmosome, ciliated centrosome.

Fig. 110—First spermatocyte, later growth stage, double plasmosome, centrosomes divided and ciliated.
Fig. 111—First spermatocyte, segmented spireme, segments opening into rings.
Figs. 112–115—First spermatocyte, stages in the formation of dyads and tetrads of prophase.
Fig. 113—First spermatocyte, formation of chromatin-plasmosome.
Figs. 116 and 117—First spermatocyte, early metaphase.
Fig. 118—First spermatocyte, equatorial plate, 31 chromosomes.
Fig. 119—First spermatocyte, metaphase spindle.
Fig. 120—First spermatocyte, anaphase spindle.
Fig. 121—First spermatocyte, telophase.
Fig. 122—First spermatocyte, telophase, polar view.
Fig. 123—Second spermatocyte, arrangement for second division.
Fig. 124—Second spermatocyte, equatorial plate, 31 chromosomes.
Fig. 125—Second spermatocyte, metaphase spindle.
Fig. 126—Second spermatocyte, anaphase spindle.
Fig. 127—Second spermatocyte, telophase.
Fig. 128—Spermatid, first stage.
Fig. 129—Spermatid, second stage.
Fig. 130—Spermatid, later stage, formation of axial filament.

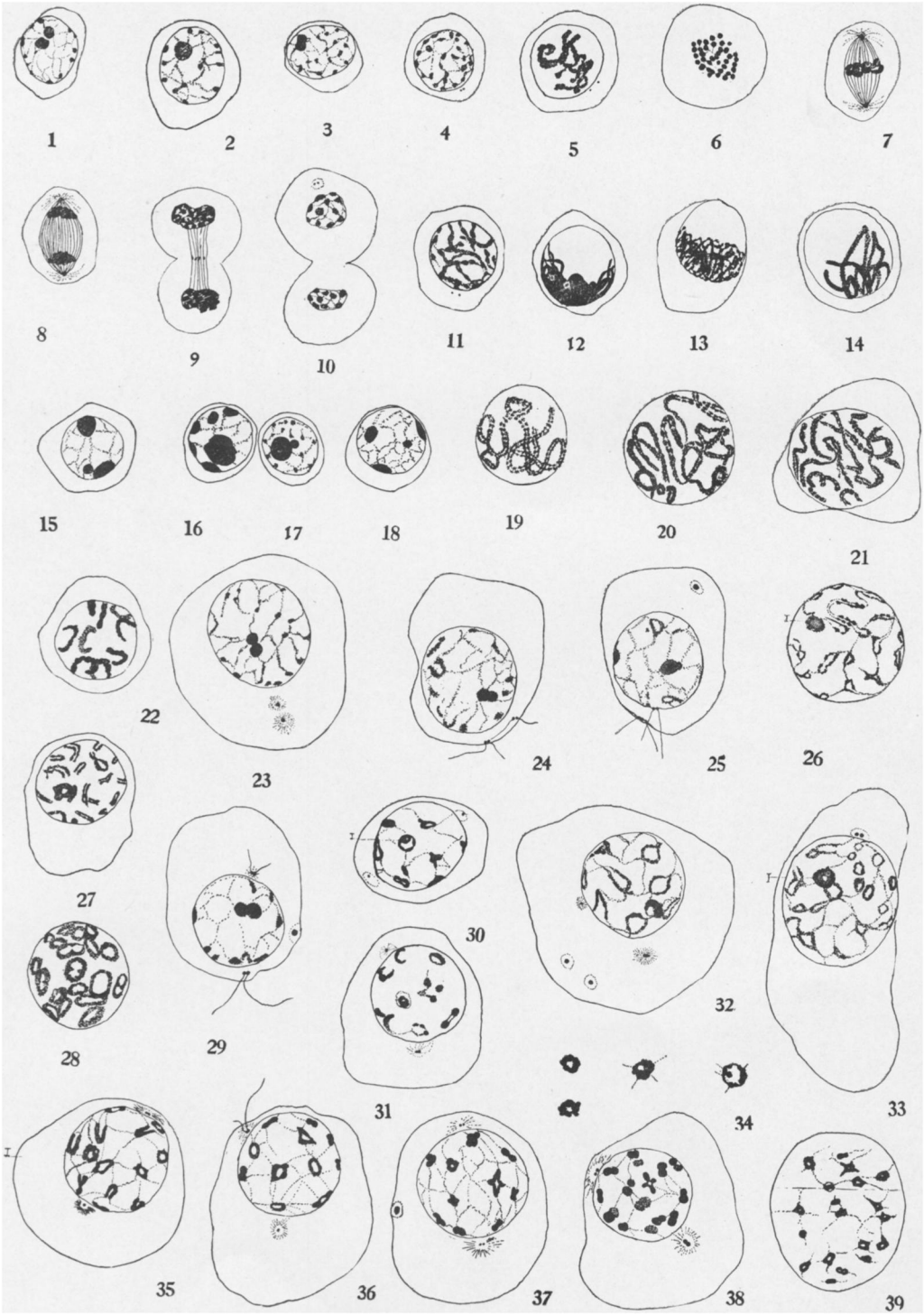
PLATE XXVI—*Automeris io*—Figs. 131–135—Spermatids, later stages, showing development.
Fig. 136—Spermatids, c. s. through axial filament and membranes.

Samia cecropia—Figs. 137 and 139—Spermatogonial stages, rest stage showing plasmosome.
Fig. 139—Spermatogonial stages, metaphase spindle.
Fig. 140—Spermatogonial stages, anaphase spindle.
Fig. 141—Spermatogonial stages, telophase.
Fig. 142—First spermatocyte, formation of spireme, preparatory to synezesis.
Fig. 143—First spermatocyte, smear showing granular thread of synezesis.
Fig. 144—First spermatocyte, early post-synapsis.
Fig. 145—First spermatocyte, later post-synapsis.
Fig. 146—First spermatocyte, late post-synapsis.
Fig. 147—First spermatocyte, growth stage, showing plasmosome and chromatin granule.
Fig. 148—First spermatocyte, growth stage, smear preparation, showing identity of chromosomes.
Figs. 149–152—First spermatocyte, formation of dyads of prophase, formation of a chromosome from a chromatin-plasmosome.
Fig. 153—First spermatocyte, equatorial plate, 30 chromosomes.
Fig. 154—First spermatocyte, metaphase spindle.
Fig. 155—First spermatocyte, anaphase spindle.
Fig. 156—First spermatocyte, telophase spindle.
Fig. 157—First spermatocyte, telophase, polar view, chromosome scattering.
Fig. 158—First spermatocyte, telophase, polar view.
Figs. 159 and 160—Second spermatocyte, arrangement for second division.
Fig. 161—Second spermatocyte, equatorial plate, 30 chromosomes.
Fig. 162—Second spermatocyte, metaphase.
Fig. 163—Second spermatocyte, anaphase.
Fig. 164—Early spermatid.
Fig. 165—Spermatid, second stage.

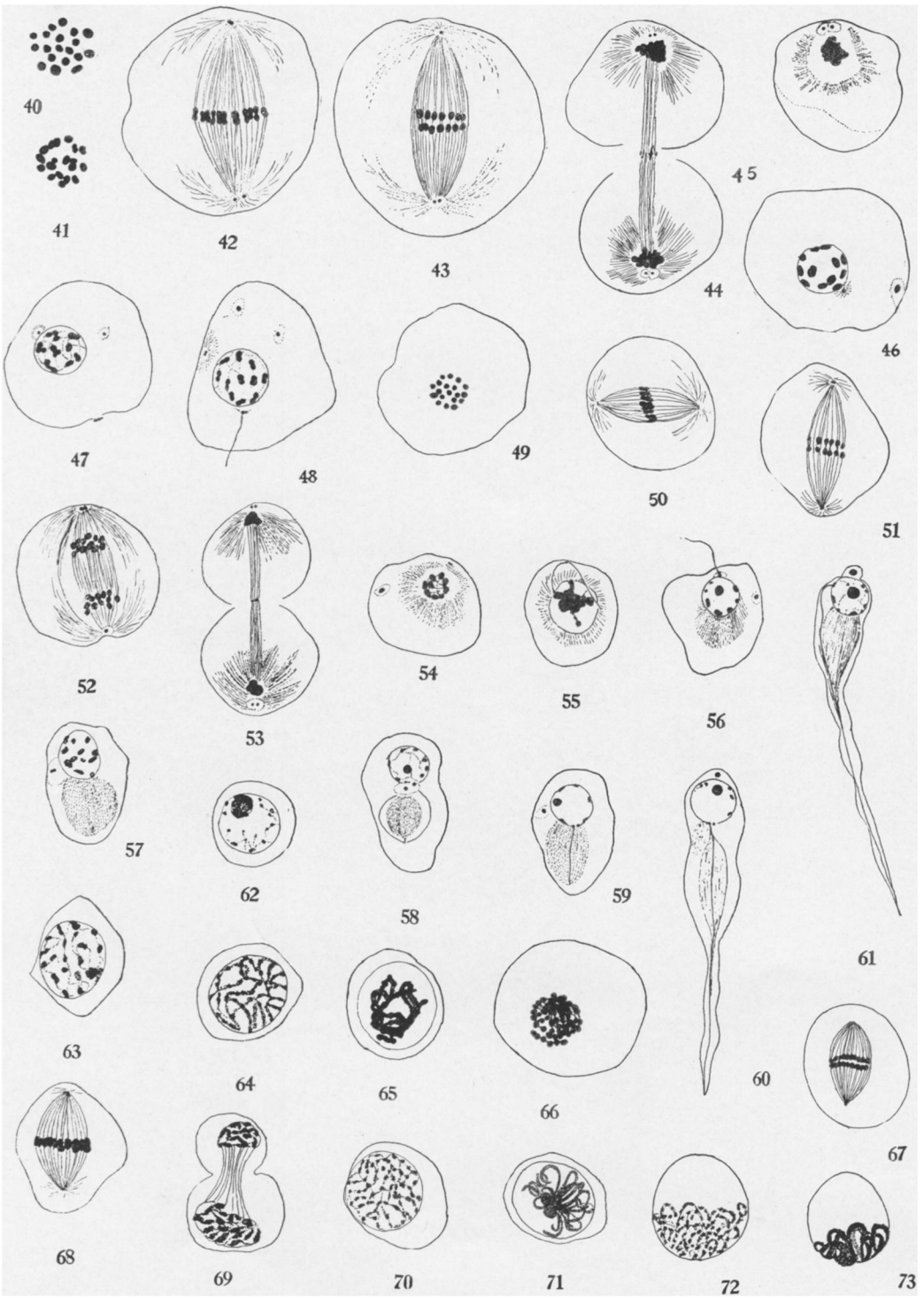
PLATE XXVII—*Samia cecropia*—Fig. 166—Spermatid, second stage.
Figs. 167–169—Spermatid, later stages.
Fig. 170—Spermatozoon, vibratile filament, ½ its length.

Acronycta sp.,?—Figs. 171 and 172—First spermatocyte, growth stage showing "chromosome granule" and mitochondria.

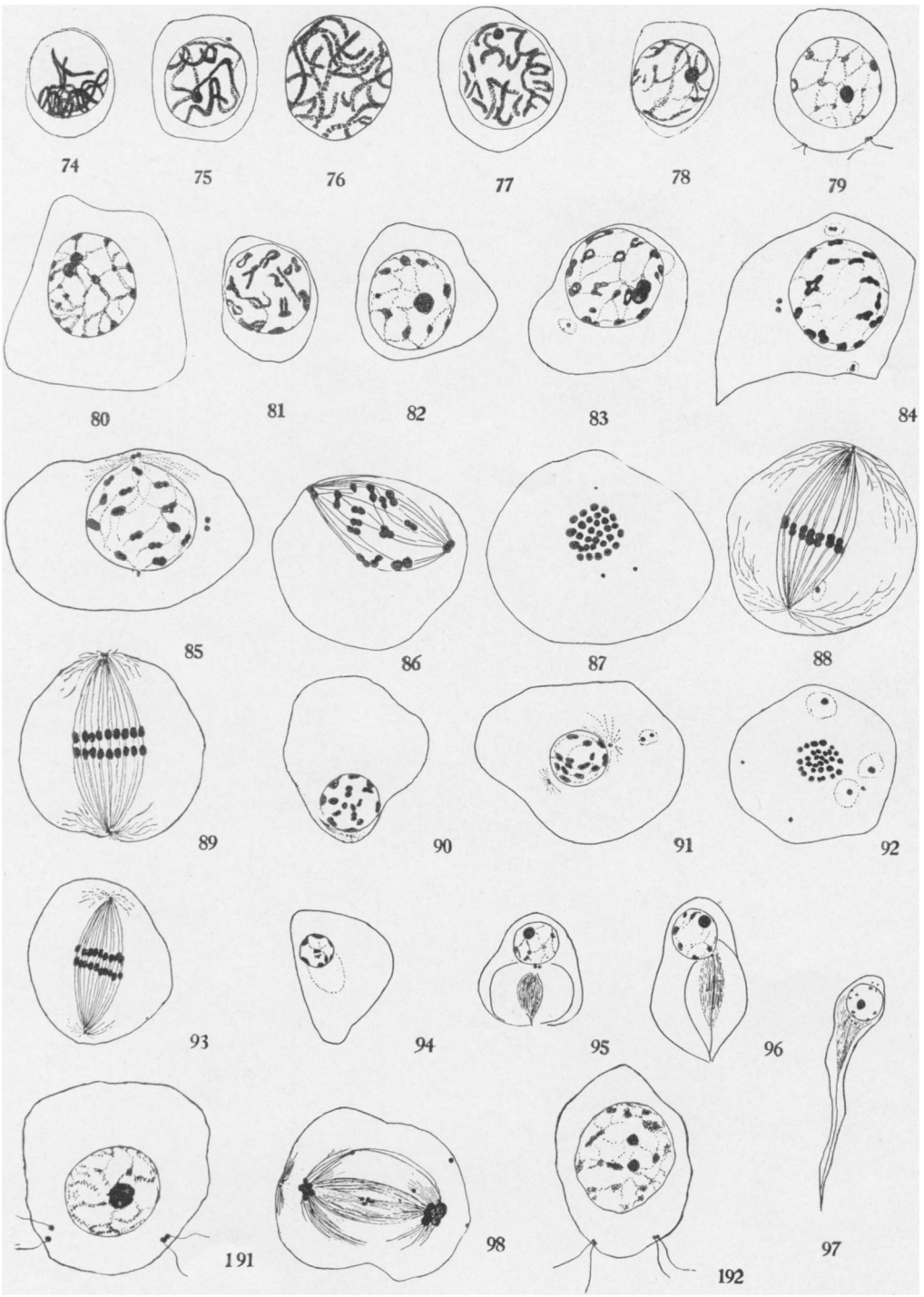
Figs. 173 and 174—First spermatocyte, stages in prophase.
Fig. 175—First spermatocyte, equatorial plate, 29 chromosomes, mitochondria and chromatin granule in cytoplasm.
Fig. 176—First spermatocyte, metaphase.
Figs. 177 and 178—First spermatocyte, anaphase.
Fig. 179—First spermatocyte, telophase.
Fig. 180—First spermatocyte, telophase, polar view.
Fig. 181—Second spermatocyte, equatorial plate.
Fig. 182—Second spermatocyte, metaphase.
Fig. 183—Second spermatocyte, anaphase.
Fig. 184—Second spermatocyte, telophase.
Fig. 185—Spermatid, second stage.
Figs. 186–190—Spermatid, development.



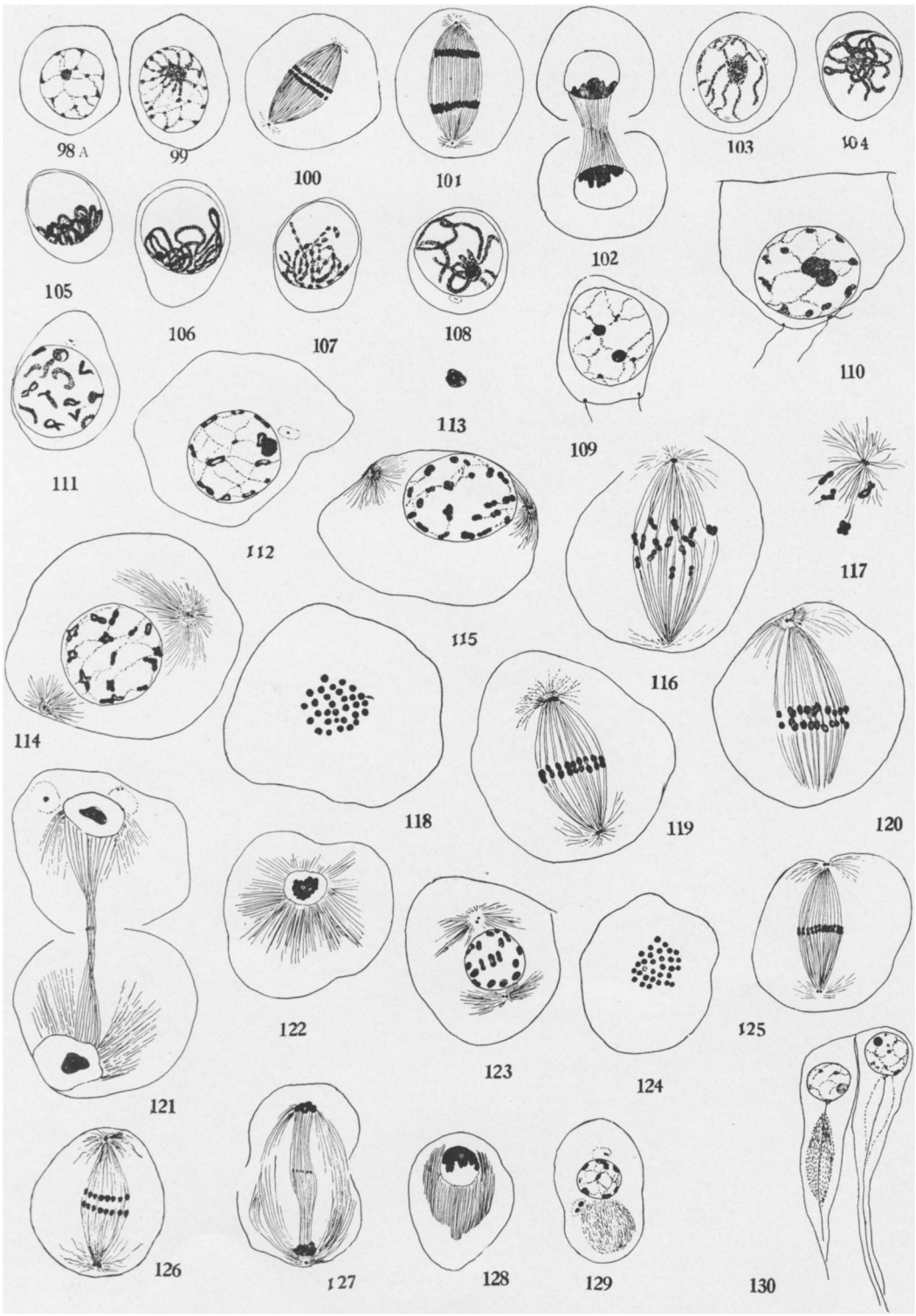
COOK: SPERMATOGENESIS IN LEPIDOPTERA.



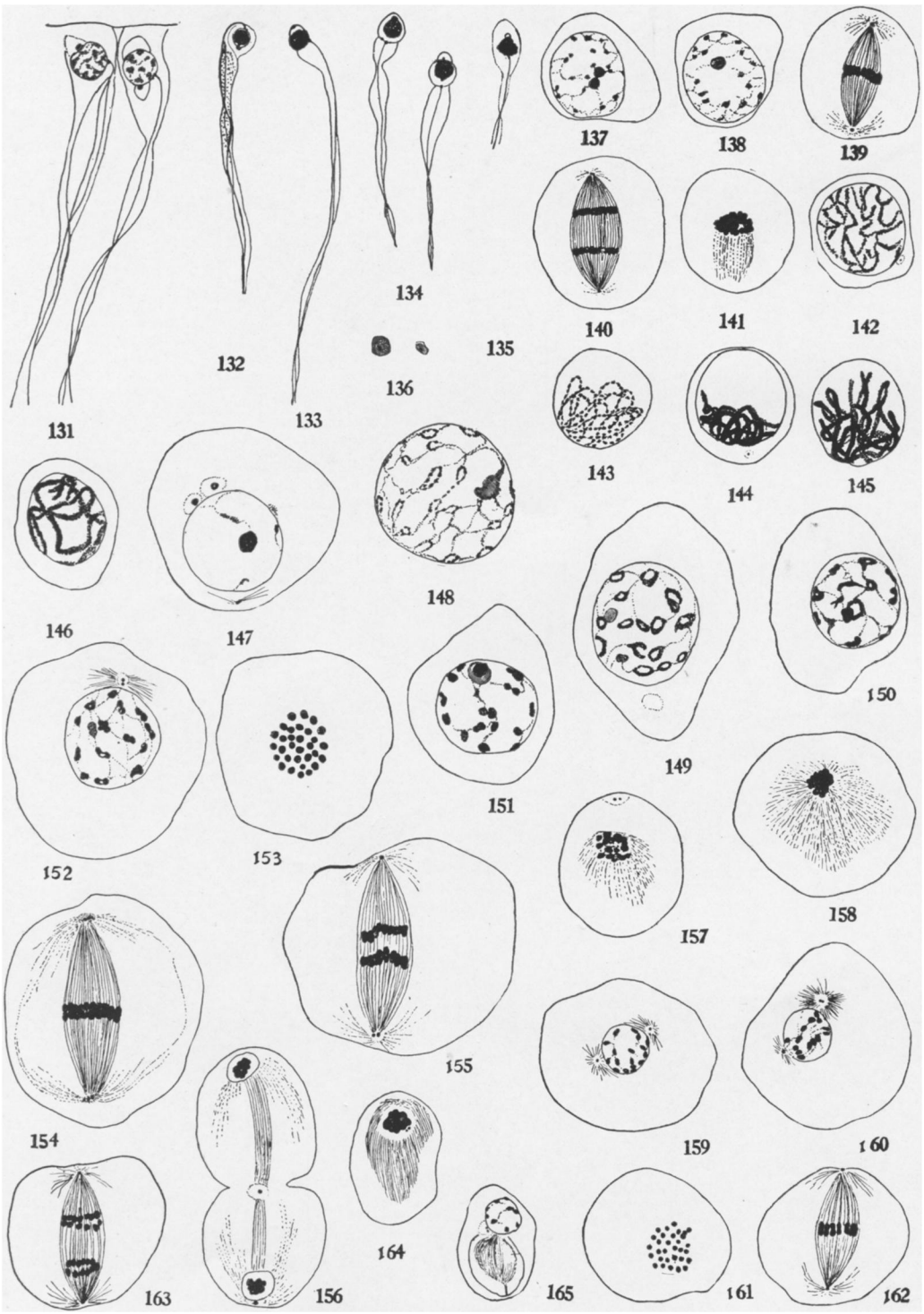
COOK: SPERMATOGENESIS IN LEPIDOPTERA



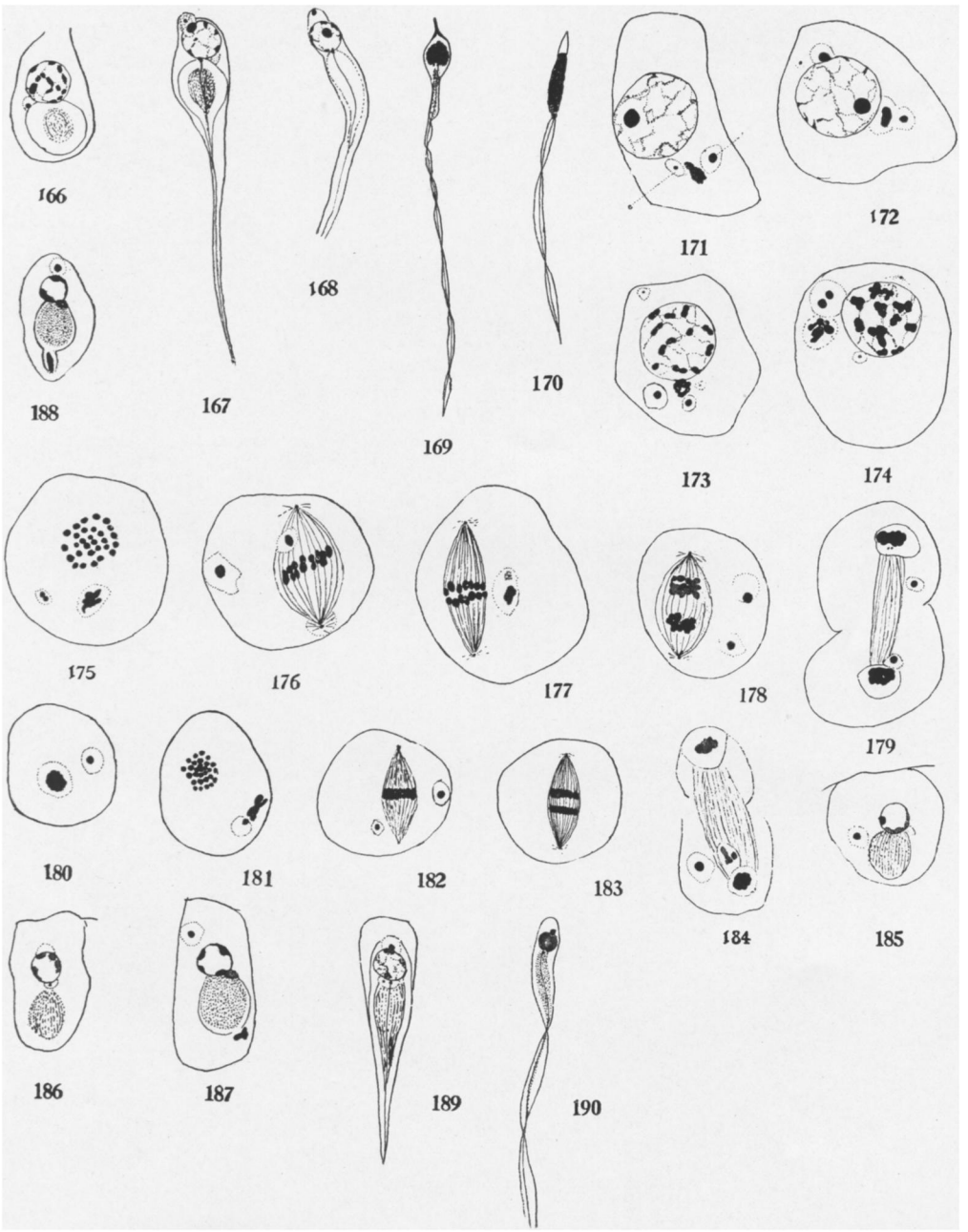
COOK: SPERMATOGENESIS IN LEPIDOPTERA.



COOK: SPERMATOGENESIS IN LEPIDOPTERA.



COOK: SPERMATOGENESIS IN LEPIDOPTERA.



COOK: SPERMATOGENESIS IN LEPIDOPTERA.