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Espermatogénesis de *Squilla oratoria* De Haan.

Después de la última mitosis espermatogonial el material cromático se difunde por el núcleo; después aparecen filamentos leptoténicos separados y distintos, los cuales se disponen por parejas al llegar el estado de la sinapsis. Los números haploide y diploide de cromosomas son veinticuatro y cuarenta y ocho, respectivamente. En el citoplasma de la espermátida aparece una vacuola que aumentando de dimensiones llega a ocupar la mayor parte de la célula, de tal modo que el núcleo se hace completamente excéntrico. El núcleo pasa por un cambio bastante complicado, hasta que finalmente se convierte en una estructura rígida y excéntrica. El núcleo pasa por un cambio bastante común y homogéneo. El centrosoma comienza a emigrar hacia el núcleo y termina por ponerse en contacto y encerrarse dentro de él, donde sufre una división y uno de los dos centrosomas hijos, que representa el centrosoma proximal, se transforma en un cuerpo en forma de bastón. El espermatozoide maduro es un corpúsculo esférico vesicular, y en uno de los polos de la esfera está situada la cabeza, que contiene dos centrosomas intranucleares.

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SPERMATOGENESIS OF *SQUILLA ORATORIA* DE HAAN

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FIFTY-ONE FIGURES (THREE PLATES)

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INTRODUCTION

The Stomatopoda has long been known to possess spermatozoa of a peculiar appearance, which differ widely not only from the ordinary filiform ones, but also from those usually found in the Decapoda. However, apart from a few rather old works, we have practically none in literature dealing with the morphology and genesis of the spermatozoon. At the suggestion of Doctor Yatsu, I undertook the study, the result of which has formed the data presented in this report. In pursuing the study as well as in working out the manuscript, I am deeply indebted to Doctor Yatsu.

LITERATURE CONCERNING THE STRUCTURE AND DEVELOPMENT
OF SPERMATOZOON OF THE STOMATOPODA

In a paper dealing with the structure of the reproductive organs of *Squilla* mantis, Grobben ('76) described for the first time the spermatozoon of the Stomatopoda. He says it is "kugelig, vielleicht etwas abgeplattet, von ganz homogener Aussehen mit sehr schmalem lichterem Band und 0.008–0.0085 Mm. im Durchmesser," and, further, that if the spermatozoon be treated with acetic acid, there appears a highly refringent body, elliptical when seen en face, which he thought to be the head of the spermatozoon.

Another work of the same author published two years later ('78), concerning chiefly the male reproductive organs of the Decapoda, contains scattered accounts about the structure of the testis of *Squilla* mantis and, further, a brief description of the development of the spermatozoon. The spermatid ('Samenzelle'), as described in that paper, has a spherical nucleus enclosing numerous nucleoli and surrounded by a faintly granular cytoplasm. At the commencement of transformation, the nuclear substance assembles toward one pole of the nucleus where the future head of the spermatozoon develops and takes a crescent shape. The substance next concentrates to form a hemispherical body which becomes homogeneous and refractive. Probably at the expense of the nuclear fluid, this body enlarges until it becomes spherical in shape and comes to lie at the center of the cell, whose cytoplasm also has now become homogeneous.

In 1885 Carnoy described the maturation divisions of *Squilla* mantis. He observed that the spireme is segmented into from twenty to twenty-four chromosomes, which arrange themselves in the equatorial plate with their longer axis parallel to the spindle axis. Each of the chromosomes splits longitudinally in the next division.

In the year following (1886), Gilson published a somewhat longer account of the spermatogenesis of *Squilla* mantis than that of Grobben referred to above. The testicular tubule is lined with a syncytial mass of cytoplasm which contains nuclei filled with clumps of chromatin. The mother-cells of the sper-

matozoon multiply exclusively by direct division. In the nucleus of those cells the spireme is formed, which is segmented into chromosomes. The sperm-cell undergoes almost no changes in external form until it becomes a mature spermatozoon. This latter process is initiated by the appearance of a small vacuole in the nucleus; this vacuole then enlarges considerably until it fills the greater part of the nuclear cavity, so that the nuclear substance comes to be pressed toward one pole of the nucleus against the nuclear membrane and assumes a cupule shape. The substance next concentrates, becomes homogeneous, and further continues to diminish in thickness for a while. Soon, however, the central part of the cupule-shaped body becomes inflated to take a button-shape and is brought into the cavity of the vacuole. In the meantime, the nucleus has a considerable growth, while the size of the cell remains nearly constant, so that it naturally follows that the cytoplasm is reduced a great deal. When, finally, the nucleus reaches full development, it fills the entire cell, the cytoplasm being completely lost, and the nuclear membrane fuses with the cell-membrane. The mature spermatozoon forms a vesicular corpuscle, spherical in shape and furnished with a very thick membrane. It contains a hyaline liquid, in which some plasmic remains may be recognized. At one pole of the wall of the vesicle the button-shaped body is attached; this generally is homogeneous, but may occasionally enclose a small vacuole. In the vas deferens the spermatozoa are agglutinated together by a cementing material into a firm cord.

More recently, ('09) Nichols described and figured the spermatozoon of *Squilla*, together with that of several other crustaceans. She says that the spermatozoon is "spherical in shape, the greater part of the cell consisting of a colorless vesicle filled with hyaline substance, while the only portion staining with methyl green is a button—or lens-shaped body at one pole of the sphere." She assumed the spermatozoon 'to forecast' that of the Decapoda.

MATERIAL AND METHODS

Squilla oratoria De Haan (*S. affinis* Berthold) is a stomatopod common along the Pacific coast of the milder districts of Japan. In the Gulf of Tokyo it is fished in great quantity throughout the year and forms a staple food. The material of the present study was obtained at intervals from November to May, 1917-'19. The males were all available for the study, since all of them supplied both early and advanced stages of spermatogenesis. To secure early stages, however, especially in spring months, it was necessary to take the hindmost region of the testis. For fixing, various reagents were tried, such as, 1) acetic alcohol; 2) acetic sublimate (glacial acetic acid in 3 per cent); 3) Bouin's fluid; 4) Worcester's fluid; 5) Flemming's strong solution, and, 6) Hermann's fluid. Of these the last two reagents were found to give the most satisfactory results and were used almost exclusively for the present study. Sections were cut from 4 to 10 μ in thickness. Smear preparations were fixed either with Bouin's fluid or with osmic vapor. For staining, Heidenhain's iron-hematoxylin was used, with or without counterstaining with orange G, Bordeaux red, Congo red, or acid fuchsin. Both safranin and Delafield's hematoxylin were found to be less satisfactory than iron-hematoxylin, and they were used only to check the above method.

MALE REPRODUCTIVE ORGAN OF *SQUILLA ORATORIA*

Grobber's description of the male reproductive organ of *Squilla mantis* ('76) applies very well to the same organ of *Squilla oratoria*. As was pointed out by that author, the organ is situated directly beneath the heart tube and above the digestive tract and hepatic organ, and consists of two distinct portions, namely, the gonad proper and the 'accessory gland.' The former lies in the posterior region of the body, ranging from the last thoracic somite to the telson, while the latter is located in the middle region, in the exposed thoracic somites. Aside from this distinction in situation, the two portions bear close resemblance with each other. Both form extremely slender

convoluted tubes, paired for the greater part of their length, only parts at the anterior extremity of the accessory gland and the posterior end of the gonad proper being unpaired. Starting from these unpaired parts, both the accessory gland and the gonad proper proceed in striking meandering courses, but largely along the longitudinal axis of the body, the former from anterior, while the latter from posterior, toward the last thoracic somite. At this somite, they both enter the penis, which is attached to the basal segment of the ambulatory leg. Somewhat before reaching the somite, the gonadal tube shows extreme convolutions, forming several complete loops in its course and presents an appearance of an entangled thread. Mature or nearly mature spermatozoa alone are contained in this part of the tube; it is consequently to be called vas deferens to distinguish it from the more posterior and less convoluted part, the testis in the strict sense. The accessory gland apparently has nothing to do with the production of spermatozoa; it contains neither mature spermatozoa nor any developmental stages of them.

ARRANGEMENT OF SEMINAL ELEMENTS IN TESTICULAR TUBE

As is shown in figure 1, the testicular tube is nearly round in cross-section and contains seminal cells representing various stages of development, save the central region where some vacant spaces are generally found. The cells are arranged in two or three distinct zones. In figure 1 three such zones are very clearly distinguishable: the outermost, made up of spermatocytes in synizesis stage dispersed with some spermatogonial and nutritive cells, another containing young spermatids, and still another with advanced spermatids. A similar arrangement exists throughout the whole length of the tube. It may be noticed that the nearer the anterior end, the older the cells are.

In a region of the outermost zone in the section of the testicular tube, perhaps along the middorsal line of the latter, is a cluster of cells (fig. 1, *pr.*) distinguishable from the neighboring parts by its characteristic appearance. It consists of a few large spermatogonial cells (*p.spg*) together with some nutritive cells (*nt*) and bulges out into the mass of seminal cells of more

advanced developmental stages. Without doubt, this cluster represents the proliferating region of the seminal tube.

A similar arrangement of seminal elements has been observed in crabs by Binford ('13) and Fasten ('18). However, in those cases, the regions containing younger elements appear in section crescentic in outline and restricted to one side of the tube wall, whereas, in *Squilla*, they form complete rings of nearly uniform thickness.

The vas deferens is lined with a rather high epithelium enclosing large nuclei. The spermatozoa contained in the cavity form a compact mass, apparently being agglutinated by some cementing material. A broad space is often found between this mass and the wall surrounding it.

SPERMATOGONIAL AND NUTRITIVE CELLS.

The transformation stages of the seminal cells up to the production of spermatids have not been traced in detail, so that I can give but a brief account of them. The spermatogonial cell (figs. 2 and 3) is polygonal and is marked off from its neighbors by a fairly distinct cytoplasmic wall. It contains a large vesicular nucleus, which is usually spheroidal or ovoid. Numerous chromatin granules of varying size are found in it. A few largest ones, occurring in the central part of the nucleus, represent the karyosomes and forms the centers of radiating linin strands, along which are arranged smaller and less conspicuous granules.

In the cytoplasm and close to the nuclear membrane, are bodies apparently to be identified as the 'chromatoid bodies' (fig. 2, *k*). They may be spheroidal, dumb-bell- or spindle-shaped and stain in basic dyes as strongly as the chromatin granules in the nucleus. Some of them are often so large and conspicuous as to be easily detected under low magnification, while some may be extremely minute and barely discernible with very high power. Small spheroidal ones look more like a centrosome, and it is rather hard to distinguish the former from the latter. The centrosome (*c*) is a single minute spheroidal body lying close to the nuclear membrane. No idiozome could be detected around it.

As was pointed out by Binford ('13) and Fasten ('18), it is possible to distinguish primary and secondary spermatogonia, although the distinction between the two is not infrequently rather obscure. The former (fig. 3) are restricted to the proliferating region mentioned above, whereas the latter (fig. 2) are distributed throughout the remaining outermost zone of the testicular tube. Apart from this topographical distinction, the two kinds of spermatogonia differ in some structural details: the nucleus of the primary spermatogonia is larger and more vesicular than that of the secondary and the karyosomes of the former are more regularly spherical.

In the spermatogonial mitoses *no continuous spireme is formed*. The chromosomes at origin are ovoid or ellipsoid in shape. In two fairly good equatorial plates (figs. 4 and 5) I was able to count forty-eight chromosomes. Between two adjoining chromosomes a linen strand is often detected. The chromatoid body (fig. 6, *k*) lies outside the spindle and enters one of the two daughter-cells without division.

Interspersed between the spermatogonial and spermatocyte cells are numerous nutritive cells (fig. 1, *nt*, fig. 7). They show usually some degree of resemblance in appearance to the spermatogonial cells, but may be distinguished by the fact that the nucleus stains somewhat darker by basic dyes and is usually irregular in shape: triangular, crescentic, sausage-shaped, or even with pseudopodia-like projections. In some cases the nucleus exhibits an appearance highly suggestive of the occurrence of amitosis. The boundary of the cell is often difficult to detect; furthermore, not infrequently a group (three to five) of nuclei are embedded in a common syncytial mass.

Among workers of decapod spermatogenesis, the origin of spermatogonial and nutritive cells has been a matter of great dispute (Fasten's paper, '14). As to *Squilla*, Grobben ('78) points out the occurrence of 'Ersatzkeim' and Gilson ('86) speaks of 'plasmodium périphérique,' both undoubtedly referring to the nutritive cells. However, concerning the question of their origins, they do not express any definite opinion. I could likewise obtain no data to decide the question, but this much

may be said with fair certainty, that the two kinds of cells under consideration have a common origin, and that no nutritive cells transform into spermatogonial cells.

GROWTH PERIOD AND MATURATION MITOSES

The chromatin of the nucleus which has undergone the last spermatogonial mitosis diffuses so completely in the nuclear cavity that the entire nucleus stains almost uniformly grayish with iron-hematoxylin. Soon, however, leptotene threads make their appearance in it. They increase gradually in thickness and staining capacity, and develop into separate threads (figs. 8 and 9). They are so numerous and overlies one another so intricately that a correct count seems hardly possible. But roughly one may say that they are forty-eight, the above-mentioned diploid number. At first, they are distributed throughout the entire nuclear cavity; soon, however, they mass together in the center of the latter, leaving a wide space in the marginal region (fig. 10). In spite of the position of the threads, this stage in *Squilla* represents the synizesis stage. According to Nichols ('02, '09), the same appears to be the case with the corresponding stages of *Oniscus*, *Hippa*, *Talorchestia*, and *Idotea*. No nucleolus, however, is found in the present form, contrary to her observations on those forms.

At the climax of the synizesis, the individual chromatin threads can hardly be made out; but somewhat prior to this there is a period in which the disposition of the threads may be fairly clearly detected. Here the arrangement of the threads in parallel pairs is very apparent; several pairs, each consisting of two threads of nearly equal length, appear in the nuclear cavity (figs. 11 and 12). Thus it seems to be clear that we have before us 'parasynapsis' instead of 'telosynapsis.' The same is also true of *Cambarus* and *Cancer* (Fasten, '14, '18) and probably of *Idotea*, *Hippa*, and *Homarus* (Nichols, '09).

When synizesis is over and the nucleus enters into diakinesis, the chromatin threads, nearly twice as thick as those of the preceding stages, come into view. In material fixed with

Hermann's fluid, stained with iron-hematoxylin and extracted fairly sufficiently, a longitudinal split is very clearly observable in each thread (fig. 13). In the pachytene stage the threads often present a distinct bouquet-like arrangement, most of them in the form of a complete loop, being arranged around a center of grouping, where they fuse with each other (fig. 14). No accounts of such peculiar arrangement of pachytene threads are found in Fasten's two papers nor in Nichols's description of the synizesis of *Homarus* and *Hippa*. But the latter author's account ('09) of the corresponding stage of *Idotea* and *Talorchestia* apparently indicates the occurrence of such arrangement. Binford's figures 10 and 11 also show the similar feature.

As the nucleus passes from the leptotene to the pachytene stage, the size of both the nucleus and the entire cell increases considerably. In the pachytene stage, which lasts for a tolerably long time, the nucleus and the cell continue to grow in some measure.

I could not determine the precise procedure by which each pachytene thread is transformed into a tetrad; I shall accordingly confine myself to a few remarks on my figures 15 to 17, which I believe to exhibit some of the successive steps passed by the threads during the change in question. In figure 15 the nucleus contains several figures, many of which are in the shape of U or V, both arms consisting of two longitudinal halves. In the next figure (fig. 16), most of these U's and V's have been transformed into hollow squares, probably by opening out of the longitudinal halves of individual arms. Lastly, in figure 17, the nucleus encloses rings, crosses, rods, and the like, indicating that the formation of tetrads has been completed. Without question, every variety of the tetrads has arisen from those squares shown above. The above observation on the tetrad formation, fragmental as it is, suggests the occurrence of more complicate processes, such as are clearly demonstrated by Wilson ('12), Montgomery ('11), and Wenrich ('16) in the spermatogenesis of some insects.

All the tetrads soon condense into rod-, dumb-bell- or crescent-shaped chromosomes, which are located mostly in the superficial

part of the nuclear cavity (fig. 18). I could not determine whether or no there is such a regularity in the orientation of particular chromosomes as is maintained by Nichols ('06) in the cases of *Oniscus* and *Porcellio*. In the stage succeeding, the chromosomes travel toward the equatorial region of the nucleus, whose membrane in the meantime vanishes and the achromatic spindle-fibers make their appearance (fig. 21). In the polar view of the metaphase (figs. 19 and 20) a few chromosomes overlies some others. It needs hardly be mentioned that the chromosomes are very large as compared with those appearing in the spermatogonial mitoses. The reduced number of chromosomes is twenty-four. The daughter-nuclei produced pass directly into the succeeding division without entering into the resting stage. In the equatorial plate of the latter division twenty-four chromosomes again seem to be found, but I could not accurately determine the number; the chromosomes are considerably smaller than those occurring in the preceding division.

The chromatoid body is visible throughout the above successive stages, virtually without undergoing any perceptible change altogether. It lies usually close to the nuclear membrane, and when the spindle is formed, it is always situated outside of the latter. Varieties of form found in spermatogonial cells are also discernible.

TRANSFORMATION OF SPERMATID INTO SPERMATOZOON

At the end of the second maturation division, the chromosomes group themselves at the poles of the spindle so closely together that they form a compact chromatin mass (fig. 22). Soon, however, clear spaces make their appearance within the mass (fig. 23), which rapidly increase in size, until the entire nucleus comes to exhibit a reticular structure. This change of the nucleus is shown in figures 23 to 26. Around the reticular nucleus is often seen a clear space devoid of granulation (fig. 26, the cell on the left side). The chromatoid body is still present.

The nucleus maintains the reticular state for a fairly long time; then some of the meshes of the network coalesce and

undergo rapid development, to produce a single large mesh which presses the remaining part of the network against one pole of the nucleus into a crescent-shaped mass (fig. 27). This stage is very frequently met in the course of transformation of spermatid; in figure 1 the middle zone consists of this stage. Both Grobben ('78) and Gilson ('86) mention the occurrence of a stage in which nuclear material assembles at one side of the nucleus and assumes a crescent shape. This apparently refers to the stage just described.

When further change sets in, the chromatin substance begins to diffuse into the single large mesh, so that the latter diminishes gradually in size (figs. 28 to 30) until finally it disappears altogether and the nucleus comes to stain uniformly dark (fig. 31). During the progress of the change, the size of the entire nucleus diminishes somewhat, and a clear space develops around it. Soon, however, the nucleus enlarges, its consistency reduced, and its power of taking stains decreases, so that it comes to stain grayish in iron-hematoxylin (figs. 32 and 33). The successive transformation stages of the spermatid nucleus described above may be found in a single slide and is not very hard to follow. Similar changes were traced by Spitzchakoff ('09) in the spermatogenesis of *Leander*; thus my figures 27 and 28 apparently correspond with his 5 to 7, my figures 29 and 30 with his 8 and 9, and my 32 and 33 with his 10, respectively.

The spermatid (fig. 33) is now polygonal and contains a spheroidal nucleus at the center. The nucleus appears finely granular and has a rather loose consistency, its appearance somewhat reminding one of a ball of wool. The outline of the nucleus is often rather indistinctly defined. In cytoplasm is a mass with a granular appearance representing the 'mitochondrial body' (*m*) of authors on the spermatogenesis of the Decapoda. The body lies, in the majority of cases, where there is the greatest space between the nucleus and cytoplasmic wall, presenting frequently a crescent shape and stains with basic dyes usually somewhat more weakly than the nucleus. The cytoplasm further contains the chromatoid body (*k*), which has nearly the same appearance as seen in preceding stages. Usually only one

body occurs; rarely two larger ones and a few additional minute ones may be present. Around the body is a narrow clear space which is especially marked when the body is imbedded within the mitochondrial body. The centrosome (*c*) is also near the nucleus; it is a minute body with no idiozome-like structure around it.

The spermatid retains the above state for some time. Further transformation is initiated by a peculiar movement of the centrosome, which starts to wander toward the nucleus. It soon comes in contact with the nuclear membrane and further makes its way into the interior of the nucleus (fig. 34), until it is enclosed within the latter (fig. 35). The nuclear membrane seems to afford but slight hindrance to this movement of the centrosome and to give way very readily to it. The centrosome lies at the centre of a clear space in the nucleus, developed apparently by recession of the nuclear substance under the influence of the body.

While the above change is going on, a vacuole makes its appearance in the cytoplasm (fig. 36, *v*). This may occur somewhat before the centrosome becomes enclosed within the nucleus, more frequently, a little later than the latter change. Sometimes two or more vacuoles appear simultaneously (fig. 40), but soon they coalesce into a single one. The vacuole then enlarges markedly, until it occupies by far the greater part of the cell, so that the nucleus becomes quite eccentric in situation and takes a hemispherical shape (figs. 37 and 38). The mitochondrial body is located on the side opposite the vacuole and lies over the nucleus, assuming the shape of a cupule. The above change in the cytoplasm may be initiated by the appearance of a clear space along one side of the nucleus, which space enlarges subsequently to arrive at the same result. Meanwhile, the nucleus continues to be reduced in size, while its staining capacity is greatly intensified.

The centrosome now undergoes a noteworthy modification. The body, which has grown somewhat in size within the nucleus, is divided into equal halves (fig. 39). Both are imbedded in a common clear space (fig. 41), or each in its own space (figs. 40

and 42). Rarely, this division of the centrosome is completed before any trace of vacuole can be detected in the cytoplasm (fig. 41).

The nucleus next undergoes an important change, both in shape and in consistency. To this time the nucleus has been hemispherical in shape, granular in consistency, and stained very heavily with iron-hematoxylin quite dark, but it now becomes ovoid (figs. 43 and 44) and then hemispherical, but with its flat surface away from the center of the cell, contrary to the initial state (figs. 42, 47 to 51). It becomes, moreover, quite homogeneous in consistency and stains with iron-hematoxylin a tawny color, save the marginal part which is still dark (figs. 46, 49 to 51). This change of consistency advances from the peripheral part centrad, as is clearly shown in figure 43, in which the central region of the nucleus is still dark and granular, while the marginal part has already become homogeneous. The nucleus in the meantime comes into intimate contact with the cell wall adjacent to it, where a low conical prominence develops from the nucleus, presenting a granular appearance, especially in its basal part (figs. 44, 47 to 51 *p*). Without doubt, this prominence represents the perforatorium. But it is somewhat doubtful if it does develop actually from the nucleus, inasmuch as there is possibility of the cytoplasm taking part of its formation. Since the prominence arises where the nucleus is in intimate association with the cell wall, it is not easy to ascertain the fact definitely. For the present, after having gone over tolerably many preparations, I am inclined to claim the nuclear origin of it.

Just before this change of the nucleus, one of the two centrosomes elongates to assume a rod-shape and takes the position of the axis of the perforatorium (figs. 47 to 51, *c.1*). The clear space around that centrosome is not infrequently discernible after the transformation (figs. 49 and 50), but later it appears to fade away. Figures 44 and 45 illustrate the change of the centrosome just mentioned. In figure 44 the centrosome is in the course of transformation at the distal extremity of the nucleus, which is ovoid in shape; around the two centrosomes is a

clear space common to both. Next, in figure 45, the centrosome has already completed its transformation, while associating with its fellow and before being imbedded completely within the nucleus; such however, is apparently to be regarded as an abnormal case. Judging from what is represented by these two figures, but particularly by the latter, it is very evident that the centrosome converted into rod-shape is to be identified as the proximal centrosome and not the distal as in the decapod spermatozoon.

Contrary to the proximal centrosome, the distal one (*c.* 2) undergoes virtually no change at all. It is placed usually in the center of the homogeneous part of the nucleus (figs. 47 and 51), but may not infrequently be quite eccentric in position in that part or may be situated just upon the boundary between that part and the perforatorium (figs. 49 and 50), or even within the latter (fig. 48).

The nucleus further diminishes its size, whereas the entire cell enlarges and its wall thickens, until finally the mature state is attained (fig. 51).

By the stage shown in figure 39 the mitochondrial body forms a cupule-shaped mass lying over the nucleus; when the nucleus comes in contact with the cell walls it assumes a ring shape and takes the position between the nucleus and the vacuole in the cytoplasm. As the transformation of the spermatid proceeds, the body becomes less significant, and in the mature spermatozoon it is no longer visible. It is not at all clear what part the body contributes to the formation of the spermatozoon, save the fact that it seems not impossible that it might constitute a part of the wall of the cytoplasmic vesicle. The chromatoid body is very easily recognizable in almost all immature spermatozoa (figs. 47 to 50). It may lie where there is some remnant of cytoplasm, either in the region directly adjacent to the nucleus or very distant from it. This body likewise appears to play no important rôle whatsoever in the formation of the mature spermatozoon, and in the latter it is no longer visible. It is not clear whether the body degenerates in situ or is expelled to the exterior from the spermatozoon, as was observed by Wilson ('13) in *Pentatoma* and by Fasten ('18) in *Cancer*.

Figure 51 represents a mature spermatozoon. It is a spherical vesicular body and bears a lens-shaped nucleus, the head, at one pole of the sphere. The size of the spermatozoon is from 9 to 11μ in diameter, while the head measures from 4 to 4.5μ in transverse and 2.5μ in vertical diameters. The membrane of the vesicle is fairly thick and appears to be somewhat resistant against pressure. Its outer surface seems to be covered with some glutinous matter, since the mature spermatozoa forms a compact mass in the cavity of the vas deferens. The vesicle contains a hyaline substance which coagulates when fixed, and then presents a faintly granular appearance. The head consists of, 1) a main part, homogeneous in consistency; 2) a subordinate conical part of granular appearance, representing the perforatorium; 3) a rod-shaped body standing in the axis of the perforatorium and derived from the proximal centrosome, and, 4) the distal centrosome imbedded within a vacuole occurring in either parts 1 or 2, or on the boundary of these two. The head is highly refringent and appears very compact. It is, moreover, very resistant and hard to disintegrate, although often its shape changes to some extent by fixation. Both Gilson ('86) and Nichols ('09) recognized that the mature spermatozoon of *Squilla* is a spherical vesicular body, at one pole of which is attached the lens-shaped or button-like head. No accounts however, are to be found about the more minute structure of the head, especially that of the centrosome and the body derived from it.

GENERAL DISCUSSION

a. Synapsis

Recently, ample evidence has been accumulated in favor of the occurrence of parasynapsis, instead of telosynapsis, through cytological studies upon various forms of animals. Especially works by such authors as Grégoire ('10), Montgomery ('11), Wilson ('12), and Wenrich ('16) seem to have demonstrated in the clearest way the validity of that view. As also in higher crustaceans, Fasten ('14, '18) maintains 'parasynapsis, and his

evidence for it derived from his study of the spermatogenesis of *Cambarus* and *Cancer* seems to be fairly convincing. Likewise, in the present material I was able to trace fairly well the successive changes of chromatin threads from the leptotene to the pachytene stage, and, notwithstanding that my observation is regrettably imperfect as to the important phenomenon of the tetrad formation, there seems to be hardly any room for doubting the existence of parasynapsis for the present case.

b. Comparative study of spermatozoa of the Decapoda and the Stomatopoda

As has already been mentioned, Nichols ('09) has pointed out that the spermatozoon of *Squilla* "seems to forecast the spermatozoon of the Decapoda." It is true that the spermatozoon shows at first glance a marked resemblance to that commonly found in the Decapoda, especially in forms belonging to the group Reptantia. However, to decide the question how far this apparent resemblance has to do with affinity in a real morphological sense, a more careful study is necessary. On the morphology and development of the decapod spermatozoa much is known through works of Grobben ('78, '06), Gilson ('86), Brandes ('97), Labbé ('03, '04), Koltzoff ('03, '06), Andrews ('04), Nichols ('09), Retzius ('09), Binford ('13), Reinhard ('13), and Fasten ('14, '18). An excellent review of the literature occurs in Fasten's earlier work ('14).

The above resemblance is mainly due to the fact that the sperm tail is replaced by a cytoplasmic vesicle which gives the entire spermatozoon an appearance radically distinct from the ordinary filiform ones. The vesicle arises in both spermatozoa from a vacuole appearing in cytoplasm and enlarges to occupy by far the greater part of it. Thus far the vesicles occurring in the two kinds of spermatozoa are in agreement. In the decapod spermatozoa the vesicle contains a substance staining rather deeply with various dyes and, according to Koltzoff ('03, '06) and Reinhard ('13), it arises by the accumulation of a kind of granule called by them 'Kapsel- or Schwanzkörnchen.'

In the mature state of the spermatozoa the vesicle acquires a firm consistency, probably by changing its nature into chitin, and it appears refringent. Internally, it contains two compartments, namely, an outer and an inner, called by Fasten ('18) 'first or primary vesicle' and 'second or secondary vesicle,' respectively. In the *Squilla* spermatozoon, on the other hand, the vesicle is simple, not divided into compartments, and contains no centrosome; its enclosure takes almost no stain at all throughout all stages of the formation of the vesicle.

A no less important distinction is to be found in the head or nucleus. In the Decapoda it appears to have a rather loose consistency and often stains but weakly. Thus in the crab spermatozoon Binford ('13) has pointed out that it is rather difficult to distinguish the nucleus from the cytoplasm in which it is imbedded. This is in sharp contrast to the fact that in *Squilla* spermatozoon the nucleus acquires a consistency entirely different from that of the cytoplasm and is marked off very clearly from the latter. As a matter of fact, this consistency of the sperm head reminds one of that of the vesicle of a decapod spermatozoon. Moreover, the head encloses the bodies derived from the centrosome just as it is in the vesicle of the latter spermatozoon. Singularly enough, the head of the *Squilla* spermatozoon shares such important features with the vesicle of the decapod spermatozoa.

There remains to be considered the structures arising from the centrosome. Many authors on the spermatogenesis of the Decapoda claim that one of the two centrosomes produced by division of the original one becomes rod-shape to form the 'central body' within the secondary vesicle, while the other maintains that it retains its initial state at the base of its fellow. In the present material, too, one centrosome becomes rod-shape, while the other undergoes no perceptible change. The question naturally arises as to the homology of the two centrosomes of *Squilla* with those of the decapod spermatozoa. As has been shown above, in *Squilla* the centrosome which takes the rod shape is the one which is more approximate to the nucleus, while the other, remaining unmodified, is the distal centrosome

lying more apart from the nucleus. In the decapod spermatozoa, on the other hand, the centrosome undergoing transformation is invariably the distal one and that which does not, the proximal centrosome. In short, the resemblances of the spermatozoa of the two groups, striking as they are at first glance, seem in large measure to be of a rather superficial nature than of a strict morphological significance.

It may be added that a case somewhat suggesting the change of the proximal centrosome in the *Squilla* spermatozoon is afforded by the spermatogenesis of *Gammarus* (Köster, '09, '10). According to the accounts of that author, the proximal centrosome placed at the base of the sperm head sends out a fiber through the head toward the perforatorium and becomes united with the latter.

SUMMARY

1. In the testicular tube the seminal cells are arranged in two or three sharply defined zones.
2. Among the spermatogonial cells two kinds may be distinguished, namely, the primary and the secondary.
3. The nutritive cells probably have a common origin with the spermatogonial cells. The view that the latter may be transformed from the former seems to be erroneous.
4. The number of chromosomes appearing during spermatogonial divisions is forty-eight.
5. After the last spermatogonial mitosis the chromatin material is diffused into the nuclear cavity; the leptotene threads make their appearance from this uniform ground; they are separate from the beginning.
6. In the synizesis stage the chromatin threads aggregate together in the central region of the nucleus.
7. In the synapsis stage the chromatin threads fuse in parallel fashion, or, in other words, the fusion is carried out parasynaptically.
8. Through each pachytene thread a longitudinal split is distinctly discernible and the threads show a clear bouquet-like arrangement.

9. How the tetrads are formed could not be determined. It is probable that the tetrad arises by two successive longitudinal splittings of each bivalent chromatin thread.

10. The number of chromosomes appearing in the division of the primary spermatocyte is twenty-four.

11. The chromatoid body may be found throughout all of these successive spermatogonial and spermatocyte stages, scarcely undergoing any modification; during mitoses it is situated outside the spindle.

12. After the division of the secondary spermatocyte the nucleus exhibits a reticular appearance and then, after going through a series of fairly complicated changes, it becomes universally granular in appearance and loose in consistency.

13. The centrosome wanders toward the nucleus and becomes completely enclosed within the latter.

14. A vacuole appears in the cytoplasm and enlarges to occupy by far the greater part of the cell, so that the nucleus becomes quite eccentric in situation, and finally comes into contact with the cell membrane.

15. The centrosome within the nucleus is divided into two halves, of which one representing the proximal centrosome becomes rod-shaped, while the other, representing the distal centrosome, remains unmodified.

16. Hand in hand with the changes of the centrosome, the nucleus increases its consistency and stains very heavily, and, further, appears entirely homogeneous and compact.

17. A conical prominence representing the perforatorium develops from the nucleus at the point where this is in association with the cell membrane, and the rod-shaped proximal centrosome takes the axial position in it.

18. In the spermatid are a chromatoid body and a mitochondrial mass; they do not appear to play any important part in the formation of the mature spermatozoon.

19. The mature spermatozoon is a spherical vesicular corpuscle, at one pole of which the head is attached; the head consists of two distinct parts, namely, a main part with homogeneous consistency and a subordinate part, or the perfora-

torium, represented by the conical prominence; within the head are enclosed the two centrosomes, of which the one standing in the axis of the perforatorium is rod-shaped.

20. The resemblance of the spermatozoon of *Squilla* with that of the Decapoda and particularly of the forms belonging to the Reptantia is of superficial rather than real morphological nature.

LITERATURE CITED

Works marked with * were not accessible to the author

- ANDREWS, E. A. 1904 Crayfish spermatozoa. *Anat. Anz.*, Bd. 25.
- BINFORD, R. 1913 The germ cells and the process of fertilization in the crab, *Menippe mercenaria*. *Jour. Morph.*, vol. 24.
- BRANDES, G. 1897 Die Spermatozoen der Dekapoden. *Sitz. k. Preuss. Acad. Wiss.*
- CARNOY, J. B. 1885 La cytodierèse chez les arthropodes. *La Cellule*, T. 1.
- FASTEN, N. 1914 Spermatogenesis of the American crayfish, *Cambarus virilis* and *Cambarus immunis* (?), with special reference to synopsis and the chromatoid bodies. *Jour. Morph.*, vol. 25.
- 1918 Spermatogenesis of the Pacific coast edible crab, *Cancer magister* Dana. *Biol. Bull.*, vol. 34.
- GILSON, G. 1886 Étude comparée de la spermatogénèse chez les arthropodes. *La Cellule*, T. 2.
- GRÉGOIRE, V. 1910 Les cinèses de maturation dans les deux règnes. *La Cellule*, T. 26.
- GROBBEN, C. 1876 Die Geschlechtsorgane von *Squilla mantis* Rond. *Sitz. k. Acad. Wiss., Mathem.-Naturw. Class.*, Bd. 74.
- 1878 Beiträge zur Kenntniss der männlichen Geschlechtsorgane der Decapoden. *Arb. zool. Inst. Wien*, Bd. 1.
- 1906 Zur Kenntniss der Decapodenspermien. *Arb. zool. Inst. Wien*, Bd. 16.
- KOLTZOFF, N. K. 1903 Untersuchungen über Spermien und Spermiogenese bei Decapoden, Vorläufige Mitteilung. *Anat. Anz.*, Bd. 24.
- 1906 Studien über die Gestalt der Zelle. I. Untersuchungen über die Spermien der Decapoden, als Einleitung in das Problem der Zellengestalt. *Arch. mikr. Anat.*, Bd. 67.
- KÖSTER, H. *1909 Morphologie und Genese der Spermatozoen von *Gammarus pulex*. *Diss. Marburg*.
- 1910 Morphologie und Genese der Spermatozoen von *Gammarus pulex*. *Zool. Anz.*, Bd. 35.
- LABBÉ, A. 1903 Sur la spermatogénèse des crustacés décapodes. *C.-R. Acad. Sci., Paris*, T. 137.
- 1904 La maturation des spermatides et la constitution des spermatozoides chez les crustacés décapodes. *Arch. Zool. Exp.*, ser. 4, T. 2.

- MONTGOMERY, T. H. 1911 The spermatogenesis of an hemipteron, *Euschistus*. Jour. Morph., vol. 22.
- NICHOLS, M. 1902 The spermatogenesis of *Oniscus asellus* Linn., with especial reference to the history of the chromatin. Proc. Amer. Philos. Soc., vol. 41.
- 1906 Chromosome relations in the spermatocytes of *Oniscus*. Biol. Bull., vol. 12.
- 1909 Comparative studies in crustacean spermatogenesis. Jour. Morph., vol. 22.
- REINHARD, L. 1913 Zum Bau der Spermien und zur Spermatogenese von *Potamobius leptodactylus* (*Astacus leptodactylus*). Arch. f. Zellforsch., Bd. 10.
- RETZIUS, G. *1909 Die Spermien der Crustaceen. Biol. Untersuch., Bd. 14.
- SPITSCHAKOFF, T. 1909 Spermien und Spermiogenese bei Cariden. Arch. f. Zellforsch., Bd. 3.
- WENRICH, D. H. 1916 The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes. Bull. Mus. Comp. Zool. Harvard Coll., vol. 60.
- WILSON, E. B. 1912 Studies on chromosomes. VIII. Observations on the maturation phenomena in certain hemiptera and other forms, with considerations of synapsis and reduction. Jour. Exp. Zool., vol. 13.
- 1913 A chromatoid body simulating an accessory chromosome in *Pentatoma*. Biol. Bull., vol. 24.

EXPLANATION OF PLATES

All the figures excepting figure I were drawn at the level of table with the aid of the camera under a Zeiss 1.5-mm. apochromatic objective and a compensating eye-piece no. 8 (tube length, 160 mm.). The combination affords a magnification 2350 diameters.

ABBREVIATIONS

<i>c</i> , centrosome	<i>p</i> , perforatorium
<i>c.1</i> , proximal centrosome	<i>pr</i> , proliferating region
<i>c.2</i> , distal centrosome	<i>p.sp.g</i> , primary spermatogonium
<i>k</i> , chromatoid body	<i>sp.c</i> , spermatocyte
<i>m</i> , mitochondrial body	<i>v</i> , vesicle
<i>nt</i> , nutritive cell	

PLATE I

EXPLANATION OF FIGURES

- 1 Cross-section of the testicular tube, showing the zonal arrangement of the seminal elements; there are three zones in the figure; at the upper pole is the proliferating region. $\times 200$.
- 2 Secondary spermatogonia; in the cytoplasm are chromatoid bodies of varying shapes and sizes.
- 3 Primary spermatogonium.
- 4 and 5 Equatorial plates of the spermatogonial mitoses, showing forty-eight chromosomes.
- 6 Side view of the spermatogonial metaphase.
- 7 A cluster of nutritive cells.
- 8 Preleptotene stage.
- 9 Leptotene stage.
- 10 Synizesis stage.

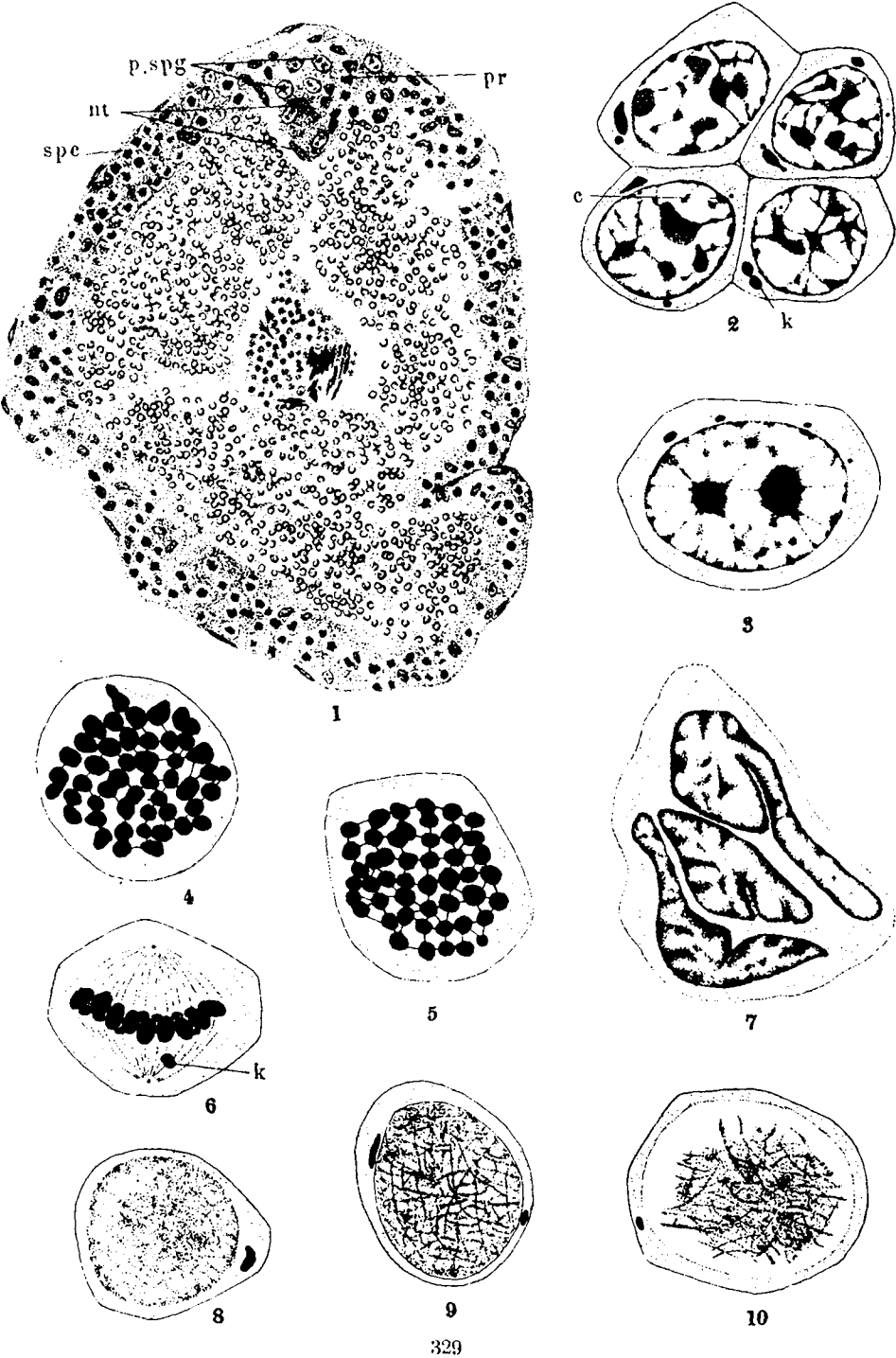


PLATE 2

EXPLANATION OF FIGURES

- 11 and 12 Synapsis stage, showing the parallel arrangement of paired threads.
- 13 Pachytene stage, showing longitudinal splittings in each thread.
- 14 Pachytene stage, showing the bouquet-like arrangement of threads.
- 15 and 16 Diakinesis stage.
- 17 Tetrads.
- 18 Tetrads transformed into rods, dumb-bells, etc.
- 19 and 20 Equatorial plates of the first maturation mitosis showing twenty-four bivalent chromosomes.
- 21 Side view of the first maturation metaphase.
- 22 to 25 Successive transformation stages of the spermatid up to just before the formation of the reticular nucleus.

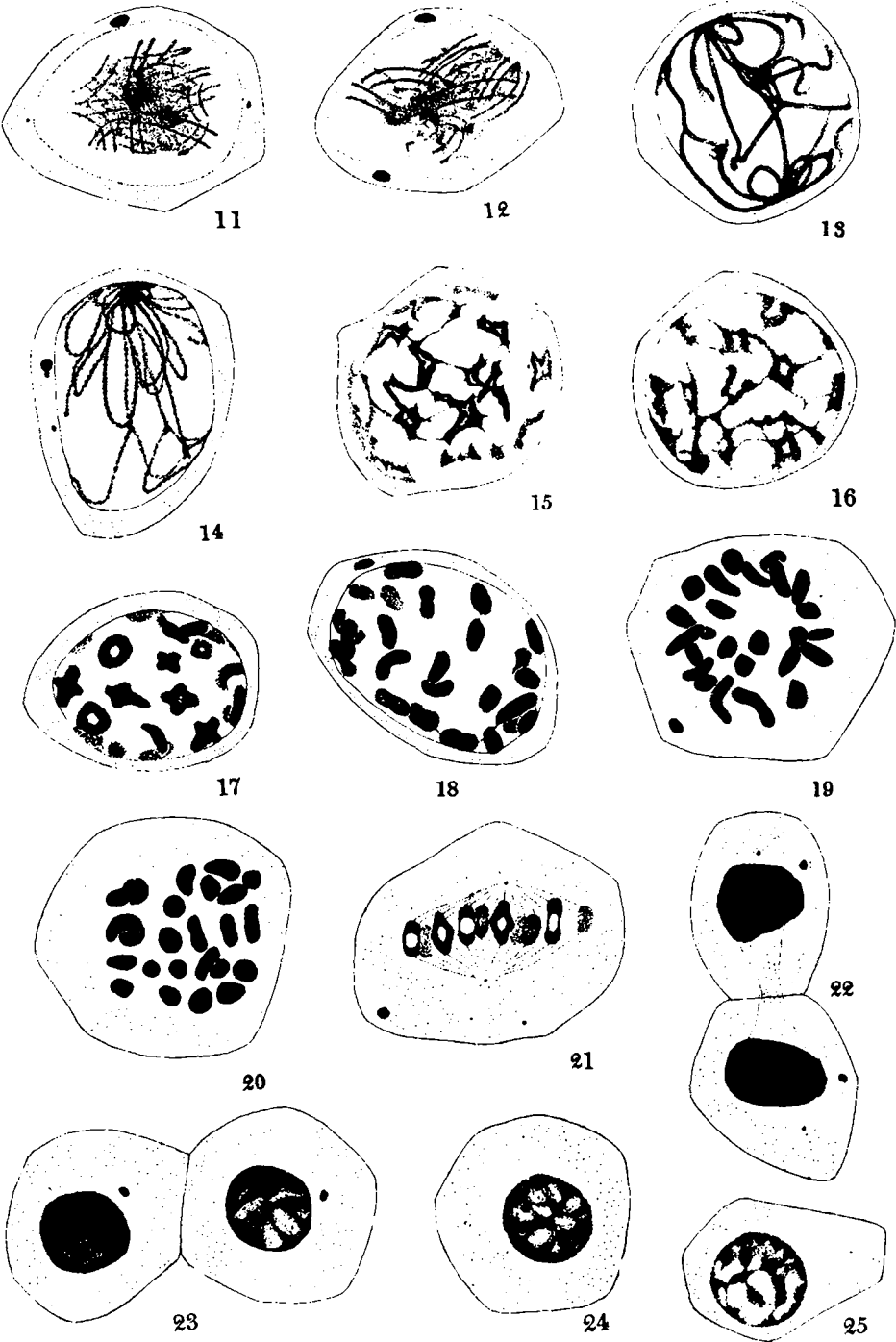


PLATE 3

EXPLANATION OF FIGURES

- 26 Spermatid with the reticular nucleus.
- 27 to 33 Successive transformation stages of the spermatid up to the formation of the nucleus with granular appearance and loose consistency.
- 34 Wandering of the centrosome into the nucleus.
- 35 Centrosome imbedded within the nucleus.
- 36 Appearance of a vacuole in the cytoplasm.
- 37 and 38 The vacuole enlarges; the nucleus diminishes its size and its staining activity increases.
- 39 Division of the centrosome.
- 40 Two daughter centrosomes lying apart from each other.
- 41 Two daughter centrosomes contained in a common clear space.
- 42 Change of the shape of the nucleus.
- 43 Consistency of the nucleus changing into homogeneous, the change advancing from the peripheral part centrad.
- 44 Change of consistency of the nucleus completed; the proximal centrosome undergoing transformation.
- 45 Proximal centrosome transformed into rod shape while associating with its fellows.
- 46 Spermatid of about the stage shown in figure 44 viewed from the top.
- 47 to 50 Nearly mature spermatozoa; the proximal centrosome lies in the axis of the perforatorium; the vacuole containing the distal centrosome lies in figure 47, within the main part of the head; in figure 48, within the perforatorium, while in figures 49 and 50 on the boundary between the two; in figures 47 and 48 the head shows a granular appearance, while in figures 49 and 50 it is perfectly homogeneous; in cytoplasm is a chromatoid body.
- 51 Mature spermatozoon.

