COMPARATIVE STUDIES IN CRUSTACEAN SPERMATOGENESIS.

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WITH 3 PLATES AND 12 TEXT FIGURES.

This study was undertaken in order to determine how far types of the class Crustacea, showing in the adult condition well marked morphological differences, would also present in the development of the germ-cells characteristic dissimilarity. Accordingly, an examination was made of the following forms: the Amphipod, *Talorchestia longicornis*, the Isopods, *Oniscus asellus* Linn. and *Idotea irrorata*, the Decapods, *Hippa talpoides*, *Astacus fluviatilis* and *Homarus vulgaris*.

There is much variety among the Crustacea with regard to the appearance of the spermatozoa and an increasing complexity of structure from the lower to the higher forms. A comparison of the spermatozoa makes, therefore, a convenient beginning for a study of this nature.

SPERMATOOA.

Entomostraca. Phyllopoda. Zacharias (1893) has studied the spermatozoa of the Daphnid *Polyphemus*. They are amœboid in shape, the character of the pseudopodia varying with the nature and density of the solution in which they are placed. In a cell so simple, of course no distinction can be made between head, middle-piece and tail. (Text Fig. 1.)

Cirripedia. In the spermatozoa of *Lepas anatifera* L. and *Balanus improvisus* Darw. Ballowitz (1908) was unable to discover any part taking the characteristic nuclear stain of alum carmine. The cell by teasing, however, could be separated into fibrils one of

which stained intensely with gentian violet. It seems probable that this represents the head, the pale fibres the tail. (Text Fig. 2.)

Copepoda. McClendon (1907) describes the spermatozoon as very similar to those of the Cirripedia.

Malacostraca. Arthrostraca (Edriophthalmata). Those which have been studied possess the flagellate type of spermatozoon. The head and tail are sharply marked off, although sometimes no middle-piece can be distinguished. Relations of size and position between head and tail differ somewhat in the members of the group. A simple rounded head followed by a tail of moderate length, and in Talorchestia by a middle-piece, characterize the Amphipoda, while in the Isopoda both head and tail are elongated, the latter excessively so. The head and tail also, instead of joining in a straight line as is the case with the Amphipoda, form with each other an acute angle, as Gilson (1886) has described it, much like the handle of a whip and its lash. The general appearance of the spermatozoa resembles that of the Cirripedia. (Text Figs. 3, 4.)

Thoracostraca (*Podophthalmata*). The spermatozoon of Mysis as described by Gilson (1886) is very like that of the Cirripedia and the Isopoda, the head and tail occupying similar relative positions, but that of Squilla is quite different, seems indeed to forecast the spermatozoon of the Decapoda. It 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. (Text Figs. 5, 6.)

Grobben (1906), Koltzoff and others have shown for the Decapoda that the more nearly related adult members are, the more nearly alike is the structure of the spermatozoa. Koltzoff (1906) especially has worked out this subject with some care and has outlined a genealogy of the Decapoda based on this feature.

The characteristic most prominent in the Decapod spermatozoon is the development of a resistant capsule in the region of the tail (*S. vesiculifera* of Koltzoff). In the lower forms this is represented by a spine-like projection (*S. anacantha*). (Text Fig. 7.) In the higher forms it is more vesicular in shape and in the region

of head or middle-piece are present projections or streamers which are entirely missing in the *S. anacantha* (Text Figs. 8-12). If these projections have their origin in the region of the neck the spermatozoon belongs to the *S. ancantina deracantha*, represented by the Galatheidæ and Paguridæ, whereas if they come from the head region it is classified as *S. cephalacantha*, represented by the Brachyura, Dromiida and Oxystomata (Text Figs. 8-12). In the latter forms the tail capsule is more or less drawn into and surrounded by the head region which gives the spermatozoon a contracted appearance and causes it to approach more nearly a spherical shape. According to Koltzoff's classification, the spermatozoa of *Astacus*, the Loricata and the Thallassemidea are placed in a separate group, the *S. contracta*, characterized by a shortening and broadening of the tail capsule. No very sharp line can, however, be drawn between these forms and the Galatheidæ and the Paguridæ, the dimensions of the tail capsule varying within comparatively wide limits.

In those Decapods which have been carefully studied the tail capsule contains a narrow tube or vesicle within which is to be found the centrosome and its derivatives. The proximal centrosome lies anteriorly in the region of the processes, while a distal, elongated and otherwise modified centrosome lies behind it extending into the tube. (Text Figs. 8, 9, 10, 12.) A comparison with the flagellate type leads to the conclusion that the nucleus may be regarded as the head, the region of the processes as the middle-piece, the capsule as the tail, while the modified distal centrosome may correspond to the axial filament. The space between the inner tube and the outer wall of the capsule is believed by Koltzoff to be occupied by an explosive substance.

The homologies of the Decapod spermatozoon have only recently been satisfactorily elucidated. Auerbach (1895) interpreted the parts of the spermatozoon much as has been outlined above, but did not work out the details. As late as 1904 Labbé described the capsule as anterior in position to the nucleus and processes and investing the acrosome. He believed it to be present only in the immature spermatozoon and that as a final stage of development the nucleus

became invaginated or surrounded in an amniotic fashion by the capsule. The latter then disintegrated, leaving as the mature spermatozoon only the nucleus, processes and "acrosome." Koltzoff, however, by his careful and detailed study of the structure and behavior of the spermatozoa has shown very conclusively that this method of homologizing the parts of the spermatozoon is consonant neither with comparative morphology nor with the behavior of the spermatozoon in fertilization.

One of the most unique and puzzling features of the Decapod spermatozoon is its lack of motility. The observations which Koltzoff was able to make on the method of fertilization throw some light

TEXT-FIGURES 1-12.

Comparative morphology of Crustacean Spermatozoa (somewhat diagrammatic).

1, Polyphemus; 2, Cirripedia; 3, Amphipoda; 4, Isopoda; 5, Mysis; 6, Squilla; 7, Carididae; 8, Anomura; 9, Anomura; 10, Homarus; 11, Astacus; 12, Brachyura.

on this subject. An examination of a mixture of the eggs and spermatozoa of *Galathea* revealed the fact that the spermatozoa are carried passively by a stream of water to the surface of the egg. When a protoplasmic process touches the surface of the egg it becomes firmly attached. The rest of the spermatozoon continues to sway back and forth until the other processes are brought in contact

with the egg, thus orienting the spermatozoon. The processes then contract until the nucleus touches the circumference of the egg. The attachment of the processes is possibly by the excretion of some adhesive substance, but also mechanical; the egg membrane is finely porous, and into the pores portions of the processes insert themselves. Koltzoff believes that the explosion of the capsule then forces the nucleus and the proximal centrosome into the egg, the derivatives of the distal centrosome and the processes being cast off. These observations lend additional weight to the homology outlined above; the capsule fulfills the function of a tail, namely, that of conveying the nucleus and centrosome into the egg.

DEVELOPMENT OF THE SPERMATZOON FROM THE SPERMATID.

Koltzoff has described with much interesting detail the development of the Decapod spermatid. He traces the origin of the middle-piece and tail from the mitochondrial granules and follows the peculiar development of the distal centrosome within the inner tube of the capsule.

Hippa is the only Decapod from which I obtained a complete series of stages illustrating the transformation of the spermatid. The fixing fluid employed, namely Flemming's strong solution, does not bring out well either the mitochondrial granules or the distal centrosome. According to Benda (1903), the proportion of acetic acid in this fluid is great enough to cause swelling and disappearance of the mitochondrial granules. It is probable, however, that in an animal so nearly related to those described by Koltzoff their development would be much the same. Of the forms described by Koltzoff, that most nearly related to Hippa is *Pagurus striatus*. Compared with this, the spermatozoon of Hippa is more contracted; both the head and capsule are broader and shorter and the demarcation between head and middle-piece is less sharp. In these respects it approaches more nearly to the *S. contracta* of the *Brachyura*. The inner tube of the capsule is closed at its distal extremity by a plug. In my preparations of Hippa the tube is much narrower than in Koltzoff's figure of *Pagurus* and in the immediate vicinity of the plug the wall of the capsule is somewhat thickened. (Pl. 3, Hi, 34.)

The reduction in size and especially in length of the spermatid in the course of its development is very marked. (Pl. 3. Hi, 29-34.) Its appearance as shown in Figs. 27-29 brings to mind the mature spermatozoon of *Homarus*, except of course for the greater relative size of the head. It is possible that there is here recapitulated a stage in the phylogeny of the spermatozoon of *Hippa*.

The spermatid goes through a remarkable series of transformations during its development. The chromatin in the nuclei of the young spermatids is arranged in bands forming a coarse meshwork with large interspaces. (Fig. Hi, 22.) The cytoplasm, at first uniform, soon shows a dense ring around the nucleus. The remainder of the cytoplasm loses the appearance of a network and no longer stains with iron hæmatoxylin. (Fig. Hi, 24.) Koltzoff, in his beautiful Figs. 1-3, Pl. XVI, shows the origin of this differentiation of the cytoplasm in the accumulation and segregation of corpuscles differing from each other in size and in staining capacity, to both of which he gives the name mitochondria. The smaller and darker granules form a mass adjoining the nucleus and correspond to the ring just described for *Hippa*. They are destined to form the middle-piece of the mature spermatozoon, while the larger and lighter corpuscles coalesce to form the tail capsule. The fixation employed for *Hippa* does not serve to bring out these granules, but the differentiation of the cytoplasm is clear. In rare instances the mitochondrial body may lie side by side with the nucleus within the cell (Fig. Hi, 23), but in general its appearance is that of Fig. Hi, 24.

Within the mitochondrial body and at one side of the nucleus arise thickened strands which extend to the periphery of the cell. They will become the streamers or projections from the middle-piece of the mature spermatozoon. As the ring increases in diameter and decreases in thickness the strands are gradually more uniformly distributed and no longer appear to arise from so circumscribed an area. (Fig. Hi, 25.) At the same time the nucleus has increased to about twice its former size, the bands of chromatin have nearly disappeared and the entire nucleus stains much more faintly.

The tail capsule has taken a more definite shape, but remains unstained. (Fig. Hi, 25a.) It next commences to elongate, a cavity

is formed in the interior, and at the distal extremity an intensely staining body is conspicuous. (Figs. Hi, 26-27.) The exact manner of formation of the inner tube is a little obscure. Koltzoff (1906) believes that the body (x) takes part in the process. Fig. Hi, 26 might lend itself to such an interpretation. During the formation of the tube only a portion of the body (x) stains brilliantly; the remainder apparently streams towards the centre of the capsule to meet that portion of the tube which is projecting from the proximal end of the capsule. Upon completion of the tube, however, the entire body stains deeply black with hæmatoxylin and becomes constricted into two unequal parts. (Fig. Hi, 27.) But this condition is not retained. The proximal portion (x) separates more completely from the distal and expands into a ring. The distal portion (x) on the other hand swells and comes to stain more diffusely. (Fig. Hi, 28.) In its later development it grows more compact, but its substance seems to retain a considerable degree of plasticity, for it presents much variety of shape. At times it appears to be partially withdrawn into the tube. (Figs. Hi, 30 a-d.) The rest of the capsule is at first quite colorless, but as it develops, longitudinal striations are formed which persist for some time. (Figs. Hi, 29-31.) Fig. 31 shows these striations as two broad, deeply staining bands. In preparations of the stage represented by Fig. 30 which have been considerably destained the striations near the surface of the capsule disappear, but in cross-sections four sharply staining dots indicate striations of the inner tube. (Figs. 30, e-f.)

The nucleus up to this point has remained nearly colorless, but it now becomes much condensed and stains vividly with saffranin, while the middle-piece takes malachite green. The capsule has become short and broad, striations have quite disappeared and the ring and plug, products of the body (x) no longer stain intensely. (Pl. 3, Hi, 32-34.)

A few stages in the development of the spermatid of *Homarus* were obtained, the material having been preserved and stained in the same manner as the material from *Hippa*. Figs. Ho, 8, 10 show two stages in the formation of the inner tube. In Fig. 8 there is as yet no wall formed marking off an inner tube within the cap-

sule. Material, however, seems to have collected close to the wall of the capsule. No ring is present at the distal end and the nucleus is condensed, flattened and surrounded by the mitochondrial substance. Enclosed in a vesicle just posterior to the middle-piece lies a densely staining body, probably the distal centrosome. In Fig. 10 the inner tube is complete, a ring has appeared at the distal end and the entire capsule has elongated. Cross sections (Figs. 9 a-b) give some idea of the manner of formation of the tube, apparently by a condensation of the capsular material which still remains connected with the outer wall by delicate strands.

Likewise in the development of the spermatid of *Eupagurus longicarpus* a few stages were studied. A "Nebenkern" appears in the youngest of them. It increases considerably in size as the nucleus stains more deeply and finally takes its position between the nucleus and the tail-capsule as the middle-piece. A densely staining globule appears at the distal end of the capsule similar to that of *Hippa*, but much smaller. The smaller size of the spermatid of *Eupagurus* at this stage is also noticeable and the more densely colorable nature of the nucleus. (Pl. 3. E, 1-5.)

Among the *Carididæ* I examined only *Palæmonetes*. The cell walls between adjacent spermatids break down and the nuclei lie in a common cytoplasm. Three such nuclei are represented in Figs. P, 1-3. Pl. 3. In the first the chromatin appears finely granular and the nuclear membrane is not sharply defined, the second is undergoing a process of degeneration and fragmentation, while the third is developing normally. The nucleus has lost its finely granular aspect and has become more or less opaque with a few ill-defined darker spots. A ring of cytoplasm has condensed about it (mitochondria) in which dark granules are scattered. In the next stage (Fig. P, 4) a portion of the ring has differentiated into a tail, between which and the nucleus two large dark spheres have arisen. Down the center of the tail also runs a slender dark filament. In the mature spermatozoon usually but one of these spheres is discoverable. In pole view (Fig. P, 6b) rays are seen to extend from it to the nucleus. The nucleus is now lens-shaped, sometimes cup-shaped. (Fig. P, 5, 6a.)

With regard to the homologies of this spermatozoon as compared with others of the same order, the dark sphere is no doubt the centrosome and the tail filament its derivative. The region in which the sphere lies is therefore the middle-piece and the striations may be compared to those visible in the spermatid of Hippa. (Fig. Hi, 24.) This spermatozoon differs from the others inasmuch as the middle-piece is not so well marked and part of the cytoplasm remains surrounding and anterior to the nucleus.

Fig. P. 7 represents an abnormal spermatid in which the nucleus has failed to condense, while the centrosomes are broken into granules.

The spermatozoa of the Isopoda are characterized by their aggregation into bundles bound together in a common sheath. Distinctness of cell boundaries is very early lost between the young spermatids. In *Oniscus* I have sometimes seen in this syncytial protoplasm bodies staining more or less darkly and showing some tendency to form threads. It seems quite possible that they correspond to the mitochondria of the Decapoda and that the long threads which soon arise in the syncytial protoplasm originate from them. In *Oniscus* I was unable to make out middle-piece and centrosome, but in *Idotea* they may occasionally be seen (Pl. 2. I, 22-25), although it is not apparent that the middle-piece arises from any previously present mitochondrial body.

The behavior of the nucleus is somewhat different in the two forms. In *Idotea* the chromatin very early leaves the center of the nucleus and is massed in irregular clumps at its periphery. (Figs. I, 19-20.) The irregular masses next merge together at one pole, while the rest of the nucleus becomes attenuated. (Pl. 2. I, 21-22.) The cap-shaped nuclear mass, as it elongates, encloses this attenuated portion of the nucleus, which is finally reduced to a mere vacuole or breaks up into a number of smaller vacuoles distributed through the substance of the nucleus. (Figs. I, 23-25.) In *Oniscus* on the other hand the chromatin is at no time massed around the periphery, but is transformed into a network of great delicacy and without vacuoles. Probably this network holds in its meshes a more fluid substance which gradually so changes its character as to stain more and more deeply. Although in both *Oniscus* and *Idotea* the nucleus

during the median stages of its development loses considerably in staining power, it never becomes so pale as in the spermatid of Hippa. (Pl. 2. O, 4-5.)

In the young spermatid of the Amphipod *Talorchestia* a small, spherical "Nebenkern" may be seen. This enlarges and elongates as the rest of the cytoplasm diminishes. Finally a differentiation is brought about into middle-piece and tail. (Pl. 2. T, 15-18.) I have no stages showing a temporary loss of staining power in the nucleus, and do not, therefore, know whether this occurs in *Talorchestia*, or whether the nucleus shown in Fig. 15 is a direct transformation of the daughter nucleus of the second spermatocytic division.

SPERMATOGENESIS UP TO THE FORMATION OF THE SPERMATIDS.

Spermatogonia.—The spermatogonia of the different forms are sufficiently unlike to permit of their being recognized without difficulty. The unlikeness is referable chiefly to the size of the nucleus itself, to the number, size, position and shape of the nucleoli, and to the manner in which the chromosomes arise.

The spermatogonia of the Isopoda resemble each other more closely than they do that of the Amphipod; also the spermatogonia of the Decapoda bear more resemblance to each other than they do to those of the Amphipoda and Isopoda. Again those of *Homarus* and *Astacus* are more like each other than like Hippa.

In *Talorchestia* the nucleolus is found close to the nuclear membrane and is often irregular in shape. More than one is frequently present. (Pl. 1. T, 1.) The nucleolus of the Isopoda has usually a slightly eccentric location and in *Idotea* is considerably larger than in *Oniscus*, although the nucleus as a whole is larger in *Oniscus*. (Figs. I, 1-2, O 1.) In the Decapoda the nucleolus is usually central or nearly so, and in all it consists of two substances staining in different degrees of intensity with iron hæmatoxylin. In *Astacus* and *Homarus* the more deeply staining substance collects around a few centres, but in Hippa it is irregularly distributed through the nucleolus. (Pl. 1. Hi, 1; Ho, 1; A, 1.) Of course the amount of granular material varies in different nucleoli and appears more abundant in darkly stained preparations.

As to the manner in which the chromosomes arise, the Decapoda and Talorchestia follow one method, the Isopoda another. In the former no spireme is found, nor even separate elongated threads; instead prochromosomal areas appear in the nucleus and the network becomes less clear and regular. The prochromosomes gradually increase in size until they are fully developed chromosomes. (Pl. 1. T, 2-3; Hi, 1-4; A, 1-2; Ho, 1-2.) The chromosomes of the Isopoda, on the other hand, arise as more or less elongated threads, a coarsening and breaking up the network as it were, which gradually shorten and condense to form mature chromosomes. Those of Idotea are generally crescent-shaped and are from the first somewhat shorter and more compact than those of Oniscus. (Figs. I, 2-3; O, 1-2.) The latter difference persists even in mitosis. (I, 4; O, 2.) The centrosome of Idotea appears to arise within the nucleus and that of Oniscus outside it, and correlated with this is a greater tendency towards polarity in the spermatogonia of Idotea, the bends of the horseshoe-shaped chromosomes pointing towards the center of the cell (Fig. I, 2.)

Synapsis.—Some difference is noticeable in the mode of transition from the last spermatogonia to the synapsis. In Idotea, Hippa and Homarus there is evidence of an approach of chromosomes in parallel pairs. (Figs. I, 5-6; Hi, 6-7; Ho, 4.) In Hippa and Homarus this is not at first accompanied by a recession of cytoplasm from the nuclear wall as is the case with Idotea. Gradually, however, the cytoplasmic connections become fewer and the chromosomes more closely associated. Those of Homarus and Astacus crowd together at one side of the nucleus, but I have not observed this condition in Hippa. (Pl. 1. Ho, 5; A, 3.) There the chromosomes mass together near the center of the cell for a very short period and then scatter more or less. During this time the members of each pair come into intimate union. Fig. Hi, 8 represents this union, Figs. 9 and 10 the immediately succeeding stages in which the members of each pair commence and continue to diverge. It is probable that a longitudinal splitting of the threads occurs at this time, although it is not always evident. The chromosomes of Idotea first appear as parallel and elongated thickenings of the nuclear network.

As the network contracts about the prominent nucleolus the ends of the chromosomes may be seen projecting from its border in pairs. (Figs. I, 5-6.) Later they shorten and approach each other more closely.

In *Talorchestia*, on the other hand, I was not able to make out with distinctness any parallel pairing of chromosomes. The first premonition of synapsis is the appearance of long, slightly thickened strands in the nucleoplasm converging towards the nucleolus. They gradually grow denser, but for some time retain their connection with the nuclear wall, while the rest of the nucleus condenses about the nucleolus. (Pl. 1. T, 5-7.) Finally, however, the entire network is converted into V-shaped chromosomes with the exception of a few delicate fibrils still stretching across to the nuclear membrane. (Fig. T, 8.)

The nucleolus of *Talorchestia* and *Idotea* is apparently more of a factor in the synaptic changes than that of *Homarus* or *Hippa*. The nucleolus of *Talorchestia* possesses considerable plasticity. Its shape is not usually truly spherical; it may be ovoid, more or less elongated or sometimes drawn out into processes not unlike the pseudopodia of an amœba. It seems to form a center around which the nuclear network contracts. In destained preparations its substance may show the color in three degrees of intensity, indicating that it is not by any means homogeneous. The nucleus of *Hippa* and *Homarus* persists throughout the synaptic changes, but is to all appearances independent of the chromatin. (Pl. 1. Hi, 11; Ho, 6.)

Maturation Divisions.—The nuclear network of the resting spermatocyte is in all delicate and stains only faintly. The condition of the nucleolus varies in the different forms. In *Oniscus* it is not very conspicuous, in *Idotea* much more so and always flattened against the nuclear membrane. In *Hippa* it is very small and there are frequently two. In *Homarus* it is rather prominent and occupies approximately the center of the cell. (Pl. 1. Hi, 14; Ho, 7; Pl. 2. I, 11.) In all it diminishes in prominence during the prophase and finally disappears.

A stronger tendency to polarity in the formation of the spermatocytic chromosomes is evident in *Hippa* than in the others, although

it is fairly well-marked in *Idotea* and also in *Astacus* (I, 12; Hi, 15-16; A, 4). It does not occur at all in *Oniscus*. Correlated with this tendency to polarity is the appearance near the center of the cell of a faintly staining body, presumably kinoplasmic in nature as it sometimes shows radiations and at other times minute granules may be seen within it. (I, 12, 13, 16; Hi, 17, 19.) In such cells also, as the chromosomes shorten and thicken, they come to lie close to the nuclear membrane, a comparatively clear space being left in the center. When the centrosomes do not arise within the nucleus, but in the cytoplasm as in *Oniscus*, the chromosomes remain distributed throughout the nucleus. (Pl. 2. O, 3.)

With regard to the manner of formation of the chromosomes, considerable similarity is noticeable between members of the same order. Thus in *Idotea*, as in *Oniscus* (Nichols, 1902), the components of each bivalent chromosome occupy with reference to each other one of three positions, end to end, side by side, or a position intermediate between these giving rise to the form of a crescent. A shape not infrequent in *Idotea*, but not noticed in *Oniscus*, is that shown in Figs. I, 13-14, c. Here the components are joined end to end and at their place of junction spread out slightly so as to form an incipient cross. This shape is not uncommon in insects and some other forms. Chromosomes like b in Figs. I, 13-15, clearly produced by the parallel approach of their components, may form either rings or crescents according to the closeness of the approximation. The chromosomes are more numerous than in *Oniscus*, it being possible to count as many as twenty-eight in the monaster stage (Pl. 2. I, 17), whereas in *Oniscus* there are but sixteen (Nichols, 1902). It is correspondingly more difficult to determine how many of each type are present, but there are without doubt at least as many as three of the parallel conjugating type. (Figs. I, 14, 17.) A side view of the metaphase gives much the same appearance as in *Oniscus* and the difference between the types is quite evident. (Fig. I, 18.) No very marked difference in size of the chromosomes can be observed in either *Oniscus* or *Idotea*, although they are not quite so nearly uniform in size as those of *Talorchestia*. In the latter animal they appear to be all of one type, formed by an end to end union; no rings

nor crescents are to be seen. Their number in the spermatocytes of *Talorchestia* is eighteen. (Pl. 2. T, 10; T, 13.)

The chromosomes of *Hippa*, on the other hand, are formed not by an end to end union of their components, but by their parallel approach. (Pl. 2. Hi, 17.) They are much more numerous than in the lower orders. I have counted sixty in the monaster of *Hippa*. (Fig. Hi, 20.) A difference in size is also a little more evident, although I have not attempted to arrange them into definite groups according to size, nor to count the number in each group.

There is always, during the prophases of maturation, an increase of cytoplasm in proportion to the nucleus. This takes place to a much greater extent in the Decapoda than in the lower orders (Hi, 14-18; Ho, 4-5), and is accompanied by the appearance in the cytoplasm of a "Nebenkern." It is no doubt correlated with the formation of mitochondria and the tail capsule of the spermatid. In the spermatocytes of *Homarus* and of *Astacus* the mitochondrial material is particularly noticeable, forming as it does dense granules in the cytoplasm (Figs. Ho, 5; A, 3.) The increase of cytoplasm in *Astacus* is accompanied by a marked growth in size of the nucleus. This can be observed as early as the synapsis and throughout the maturation stages the cells of *Astacus* are nearly twice as large as those of *Homarus*.

THE MALE REPRODUCTIVE ORGANS.

The testis of *Idotea*, like that of the terrestrial Isopoda consists of three lobes emptying separately into the vas. The testes are also alike inasmuch as within the lobes the developing germ cells do not present a continuous series but occupy zones rather sharply marked off from each other. Pl. 3. I, 28. In *Oniscus* and *Porcellio* the zones follow each other in linear fashion (Nichols, 1902), while in *Idotea* the arrangement is lateral.

The upper portion of the vas of the Isopoda is expanded. In the terrestrial forms it is appreciably wider than the lower portion and lined with large secreting cells. In *Idotea* the difference in diameter is not so great and ordinarily the cells lining the two portions do not differ much in appearance. At times, however, a few cells on

one side of the vas become greatly enlarged, and the nuclei amœboid. The nucleus at first contains granules, a few sharply staining, the numerous remainder less densely colored. It expands and fragments and the contents become finely granular, except for a few which remain somewhat larger and darker. (Pl. 3. I, 26-27.) Granules, however, do not appear in the cytoplasm, which becomes more and more vacuolated and finally commences to disintegrate. The appearance of these secretory cells is quite different from that of the land Isopoda. (Pl. 3. O, 6.) There they are far larger, the nuclear granules more nearly uniform in size, and the cytoplasm filled with similar granules which stain less intensely than those within the nucleus. (Nichols, 1902.)

The structure of the testis of *Talorchestia* is somewhat simpler than that of the Isopoda. It consists of a single follicle in which the germ cells develop in laterally arranged areas. There is no constriction between the testis and the upper portion of the vas and in longitudinal section germ cells are found on one side of the lumen, secreting cells on the other. (Pl. 3. T, 23.) The secreting cells are different in appearance from those of the Isopoda. Some of the cells contain nuclei comparatively small with the chromatin showing a decided tendency to gather in lumps close to the nuclear wall. (Pl. 3. T, 19.) The nucleus increases in size and sometimes becomes amœboid at its margin (Pl. 3. T, 20) and the chromosome granules become more distributed through the nucleoplasm. Other cells contain nuclei of greater size in which the chromatin is no longer applied to the nuclear wall, but consists of irregular masses connected with each other and with the well-defined nuclear membrane by a delicate network. (Pl. 3. T, 21.) In one cell a large vacuole was seen in the center. (Pl. 3. T, 22.) This condition is probably degenerative, since the cells adjacent to the germ cells disintegrate. (Pl. 3. T, 23.)

The testis of the Decapoda is more complicated in structure than that of the Amphipoda and Isopoda. It consists of numerous closely crowded follicles opening into a much coiled tube. Different follicles of *Hippa* contain during the breeding season different stages in the development of the germ cells and a continuous series is therefore

not difficult to obtain. The follicles of *Astacus* and *Homarus*, on the other hand, present comparatively few stages at any one time.

SUMMARY AND CONCLUSIONS.

The foregoing review and comparison shows that within the limits of the Class Crustacea differentiation of the chromatin occurs to a degree appreciable to our vision and in a way that is to a certain extent characteristic of the orders included in the class. The differentiation takes place as a divergence of form and relative size.

Thus in the Amphipod little difference is noticeable in the size or shape of the chromosomes, but in the Isopoda two types are discoverable in the spermatocytes, those in which the components are joined end to end forming rods or dumb-bells and those in which they are joined side by side forming rings, these differences being accompanied by a somewhat more marked difference in size. The chromosomes of the Decapoda, though differing from each other but little in shape, show some difference in size. On the whole, however, the Crustacea as compared with that other large class of the Arthropoda, the Hexapoda, exhibit less differentiation in the form and size of the chromosomes, nothing comparable to the accessory chromosomes having been found and none exceptionally large such as McClung has described for the Orthoptera.

As regards the number of chromosomes but little relation is evident to the degree of development. In the lower forms they may be very numerous, eighty-four (reduced number) in *Artemia* as described by Brauer (1893) or comparatively few, eleven or twelve in *Cyclops* (Ruckert, 1894). In the Decapoda they are likely to be numerous, sixty in *Hippa*, fifty-eight in *Astacus* (Prowazek, 1902), while in the Isopoda and Amphipoda there are not so many, *Oniscus* sixteen, *Idotea* twenty-eight, *Talorchestia* eighteen. Braun, however (1907), writing about the specific chromosome number of the genus *Cyclops*, draws a parallel between the reduction in number of chromosomes (from twenty-two in *C. strenuus* to six *C. gracilis*) and a gradual reduction in the development of the rudimentary swimmeret. According to his account also, in *C. viridis* two of the chromosomes are much smaller than the others and in *C. prasinus* one is smaller than the others.

The formation of the chromosomes from the nuclear network occurs in one of two ways, either around prochromosomal centers (*Homarus*, *Hippa*, *Astacus*, *Talorchestia*), or as more or less elongated loops or threads. (*Oniscus*, *Idotea*.) Of course these need not be considered as entirely distinct methods of formation. Transitions might readily occur between a condition in which there is an early determination of chromatic material towards circumscribed areas and one in which the network first breaks up into loops or strands and is later condensed.

The parallel conjugation of chromosomes during synapsis seen in *Hippa*, *Homarus* and *Idotea*,¹ and their subsequent divergence recalls similar phenomena described for such widely different organisms as flowering plants (Bergh, 1904), Nematoda (Marcus, 1906), Hexapoda (Otte, 1906; Stevens, 1906; Henderson, 1907) and Amphibia (Montgomery, 1903). Multiplication of such instances indicates that this may be the usual method of conjugation during synapsis, although in many cases obscured by the dense massing of the chromatin at this stage. This method of conjugation of course suggests a more intimate union of male and female components and an attraction between the elements of the chromosomes rather than between the chromosomes as a whole.

The tendency to polarity in the arrangement of the chromatin seen in *Idotea*, *Hippa* and *Astacus* evidently depends on the location of the kinoplasm, whether within or without the nucleus, and as the polarity is accompanied by a centrifugal movement of the chromosomes as mitosis approaches, it is a tempting hypothesis to suppose that at this period some substance is elaborated in the kinoplasmic region which sets up an outward current and that this current carries the chromosomes with it.

Relationship clearly does not determine the location of kinoplasm, for it arises within the nucleus in *Idotea* and without in *Oniscus*. To a slight degree it seems to determine the appearance and behavior of the nucleoli, but not nearly to the same extent that it affects the origin and arrangement of mitochondria.

¹Something similar has been observed for *Cyclops strenuus* by Lerat (1905).

LITERATURE LIST.

- AUERBACH, L. Spermatologische Mittheilungen: 72 Jahr. Schles. Gesell. f. Vaterl. Cult. Zool. bot. Sect., pp. 30-34, 1895.
- BALLOWITZ, E. Kopfflose Spermien der Cirripeden: Z. w. Z., 91, 3, 1908.
- BENDA, K. Die Mitochondria: Ergeb. Anat. u. Entw. gesch. 12, 1903.
- BERGH, J. Formation des chromosomes heterotypiques dans la sporogenese vegetale: La Cellule, XXI, 2, 1904.
- BRAUER, A. Zur Kenntniss der Reifung des parthenogenetisch sich entwickelnden Eies von *Artemia salina*: A. m. A., XLIII, 1893.
- BRAUN. Ueber spezifische Chromosomen-Zahlen in der Gattung *Cyclops*: Z. A., XXXII, 14, 1907.
- GILSON, G. Spermatogenese chez les Arthropodes: La Cellule, Vols. I and II, 1884-'86.
- GROBBEN, K. Zur Kenntniss der Dekapodenspermien: Arbeit. Zool. Inst. Univ. Wien, XVI, 3, 1906.
- HENDERSON, W. D. Zur Kenntniss der Spermatogenese von *Dytiscus marginalis*: Z. w. Z., 87, 4, 1907.
- KOLTZOFF, K. K. Studien über Gestalt der Zelle. 1. Spermien der Dekapoda: A. m. A., 67, 1906.
- LABBÉ, A. Maturation des spermatozoides et structure de spermatides chez les Crustacés décapodes: Archiv. Zool. Exp. et Gen. Notes, No. 1, 1904.
- LERAT, P. Les phenomenes de maturation dans l'ovogénèse et la spermatogénèse de *Cyclops strenuus*: La Cellule, XXII, 1, 1905.
- MARCUS, U. Ei- und Samenreife bei *Ascaris canis*: A. m. A. 68, 1906.
- MCCLENDON, J. F. On the development of parasitic Copepods: Biol. Bull., XII, 2, 1907.
- MCCLUNG, C. E. The Chromosome Complex of Orthopteran Spermatocytes: Biol. Bull., IX, 5, 1905.
- MONTGOMERY, T. H. 1. The Heterotypic Maturation Mitosis in Amphibia and its General Significance: Biol. Bull. IV, 1903. 2. Some Observations and Considerations upon the Maturation Phenomena of the Germ Cells: Biol. Bull. VI, 1904.
- NICHOLS, M. L. The Spermatogenesis of *Oniscus asellus* Linn., etc.: Proc. Am. Phil. Soc., XLI, 168, 1902.
- OTTE, H. Samenreifung und Samenbildung von *Locusta viridissima*: Z. A., Aug. 28, 1906.
- PROWAZEK, S. Beitrag zur Krebs-spermatogenese: Z. w. Z., 71, 3, 1902.
- RUCKERT, J. Zur Eireifung bei Copepoden: Merkel u. Bonnet Ergeb., Anat. Hefte, 1 Abt., XII, 1894.
- STEVENS, N. M. The Germ Cells of Aphids. Carnegie Inst., 1906.
- ZACHARIAS, E. Ueber die amoboiden Bewegungen der Spermatozoon von *Polyphe-mus pediculus*. Z. w. Z., XLI, 1893.

EXPLANATION OF FIGURES.

Camera drawings at the level of the table with Zeiss homogeneous immersion objective 1/12, ocular 12.

Part of the material was stained in Hermann's fluid, the remainder in Flemming's fluid. It was stained either with iron hæmatoxylin or with safranin and malachite green.

PLATE I.

Talorchestia longicornis.

- T, 1. Resting spermatogonia.
- T, 2 and T, 3. Spermatogonial prophases.
- T, 4. Metaphase.
- T, 5-9. Synapsis.

Idotea irrorata.

- I, 1. Resting spermatogonium.
- I, 2 and I, 3. Spermatogonial prophases.
- I, 4. Metaphase.
- I, 5. Early synapsis. Parallel strands appear in the network.
- I, 6. Later synapsis.
- I, 7-9. Divergence of conjugated chromosomes.

Oniscus asellus Linn.

- O, 1. Spermatogonial prophase.
- O, 2. Anaphase.

Hippa talpoides.

- Hi, 1. Resting spermatogonium.
- Hi, 2-4. Prophases.
- Hi, 5. Metaphase.
- Hi, 6 and Hi, 7. Nuclei of the last generation of spermatogonia. Approach of chromosomes in pairs.
- Hi, 8-12. Post-synapsis. Gradual divergence of conjugated chromosomes.
- Hi, 13. Final conversion of chromosomes into the network of the resting spermatocyte.
- Hi, 14. Resting spermatocyte. Nucleoli reduced in size.

Astacus fluviatilis.

- A, 1. Early spermatogonial prophase.
- A, 2. Late prophase.
- A, 3. Synapsis. Mitochondria in the cytoplasm.

Homarus vulgaris.

- Ho, 1. Resting spermatogonium.
- Ho, 2. Early spermatogonial prophase.
- Ho, 3. Very late prophase.
- Ho, 4. Spermatogonium of the last generation. Approach of the chromosomes in pairs.
- Ho, 5. Synapsis. Longitudinal conjugation of chromosomes. Mitochondria in the cytoplasm.
- Ho, 6. Divergence of the pairs.
- Ho, 7. Resting spermatocyte. Mitochondria in the cytoplasm.

PLATE II.

Talorchestia longicornis.

- T, 10. Spermatocytic prophase. 18 chromosomes.
- T, 11. Spermatocytic prophase. Origin of kinoplasm within the nucleus.
- T, 12. Spermatocytic prophase. Centrosomes within the nucleus.
- T, 13. Formation of equatorial plate. Pole view. 18 chromosomes.
- T, 14. Metaphase.
- T, 15. Young spermatid.
- T, 16-18. Gradual differentiation of head, middle-piece and tail.

Idotea irrorata.

- I, 10. Post-synapsis. Peculiar appearance of the nucleolus.
- I, 11. Resting spermatocyte or very early prophase.
- I, 12. Spermatocytic prophase. Origin of kinoplasm within the nucleus.
- I, 13-15. Spermatocytic prophases. The chromosomes are joined either end to end (a) or side by side (b). c, cross-shaped chromosomes. 14-15, surface views.
- I, 16. Later prophase. One of the chromosomes is abnormally late in condensing. Centrosomes visible within the kinoplasm.
- I, 17. Equatorial plate. Pole view. 28 chromosomes.
- I, 18. Equatorial plate. Side view. Two types of chromosomes.
- I, 19-25. Development of the spermatid.

Oniscus asellus Linn.

- O, 3. Spermatocytic prophase. Origin of centrosomes without the nucleus. Compare Figs. I, 12, 13 and 16.
- O, 4-5. Young spermatids.

Hippa talpoides.

- Hi, 15-16. Spermatocytic prophases. Longitudinal splitting of the chromatin. Increase of cytoplasm.
- Hi, 17. Later prophase. Origin of kinoplasm within the nucleus.
- Hi, 18. Later prophase. Chromosomes mostly U or V-shaped. Mitochondria in the cytoplasm.
- Hi, 19. Late prophase of the first spermatocyte. Kinoplasm within the nucleus.
- Hi, 20. Metaphase. Pole view. 60 chromosomes.
- Hi 21. Metaphase. Side view.

Astacus fluviatilis.

- A, 4. Prophase of the first spermatocyte. Polarity of chromatic loops. Mitochondria in the cytoplasm.
- A, 5. Spermatocytic metaphase.

PLATE III.

Hippa talpoides.

- Hi, 22. Young spermatid.
- Hi, 23. Abnormal spermatid.
- Hi, 24. Development of mitochondrial ring and of the strands which will become the streamers of the middle-piece.
- Hi, 25. (a) Differentiation of tail-capsule and decoloration of nucleus. (b) Pole view.
- Hi, 26-28. Development of tail-capsule, tube, ring and plug. In these figures the streamers are not shown.
- Hi, 29. Elongation of the capsule. Condensation of x^2 . Appearance of striations.
- Hi, 30. a, Later stage. b, c and d, variations in the appearance of x^2 . e and f, cross sections in the region of the nucleus and of x^2 .
- Hi, 31. a, Stage very nearly the same as 29, but of somewhat different aspect. b, cross section of x^2 .
- Hi, 32-34. Final stages in the development of the spermatid.

Homarus vulgaris.

- Ho, 8. Spermatid before the formation of the tube.
- Ho, 9. (a) and (b). Cross sections showing manner of formation of tube. (c) In the region of the middle-piece, showing the centrosome and beginnings of the three streamers.
- Ho, 10. Spermatid after formation of the tube.

Eupagurus longicarpus.

- E, 1-5. Development of the spermatid.

Palaemonetes sp.

Development of the spermatid.

- P, 1-3. Three nuclei of the syncytium. 2 is disintegrating. 3. Condensation of mitochondria around transformed nucleus.
- P, 4. Formation of tail. Two centrosomes and an axial filament are evident.
- P, 5. Cup-shaped nucleus and single centrosome. The tail has elongated.
- P, 6. (a) Lens-shaped nucleus. Beaded axial filament. (b) Pole view.
- P, 7. Abnormal spermatid.

Talorchestia longicornis.

- T, 19-22. Secretory cells from the vasa deferentia.
- T, 23. Testis and vas. go., spermatogonia. cy., spermatocytes.

Idotea irrorata.

- I, 26-27. Secretory cells from the vas.
- I, 28. One of the three testis lobes, showing the lateral arrangement of the developing germ-cell.

Oniscus asellus Linn.

- O, 6. Secretory cell from the vas. A small portion only of nuclear network shown.