CORRELATION OF THE LEVEL OF SPERMATOGENESIS TO INSTARS OF LEUCOPHAEA MADERAE (F.) (BLATTIDAE, ORTHOPTERA)

by

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INTRODUCTION

This study was undertaken as an integral part of a Public Health Service grant to study sexual dimorphism of cockroaches which is being conducted by the major professor. The correlation of the level of spermatogenesis to instar, a field which seems to have been generally neglected, was the object of this investigation.

REVIEW OF LITERATURE

Kirby (1904), stated that Leucophaea maderae (F.), the species studied in this investigation, was originally described by Fabricius in 1781, who placed it in the subfamily Panchlorinae of the family Blattidae. He described it as a cosmopolitan species of Madeira and Africa.

This cockroach is now broadly established in the West Indies, Coastal Brazil, Central America, Cuba, Jamaica, Hispaniola, Puerto Rico, and the Bahamas. Palisot de Beauvios reported it first in America in the early nineteenth century. He believed it to be originally from Africa and imported into French colonies in America. It is known from the Canaries, Morocco, Andalusia in Spain, and Corsica, doubtless as infiltrations in colonial commerce with western Africa. It is also known from Java, the Philippines, and the Hawaiian group (Rehn, 1945). Gurney (1953) reported it widely spread in commerce throughout the tropics and subtropics; an infestation in the basements of New York City apartment buildings is probably the result of an introduction from Puerto Rico.

It was found that the development of the testes of the Madeira roach conformed closely to that of the "typical" orthopteran insect as described by Snodgrass (1937). The development of a true sacculated testis was not found in nymphs earlier than late second instar.

Most of the work done on spermatogenesis has been along the lines of heredity or chromosome studies, and not in relation to rate of growth or metamorphosis. The literature contains very little reference to spermatogenetic investigations on nymphs or other immature stages. However, extensive published works on spermatogenesis in adult orthopterans present a clear picture of the mechanics of this process in these insects.

Three stages are generally recognized in the process of sperm formation. McClung (1900) discussed the stages as follows. The first is the "division period" in which the primordial germ cells, by rapid and repeated divisions, change to large numbers of cells called spermatogonia. Following is the "growth period" in which the cells do not divide, but gradually increase in size. Then the cells enter the third stage or "maturation period" in which the usual chromosome number is halved by a complicated process of meiotic division.

The apical cell of the testis follicle, its nature and its activities, have been an object of investigation for considerable time. Carlson (1945) gave good accounts of the activity of the apical cell and showed that from it the primordial germ cells derive their nourishment.

Paulmier (1899) stated that in the hemipterous Anasa, the spermatogonia were found at the blind end of the follicle and occurred singly or in groups not yet surrounded by a membrane. He also stated that following a number of divisions, each group became surrounded by connective tissue continuous with the follicular wall; this group then was called a cyst.

Cysts of spermatogonia were found by Sutton (1900) in his study on the grasshopper, Brachystola magna. He reported, however, that some of the cysts contained only two cells, which differed from the belief of Paulmier that the cyst membrane does not form until a greater number of cells are present.

McClung (1900) also found cysts of two cells while working on Hippiscus, a grasshopper.

Sutton (1900) and McClung (1900) found in many orthoptera that the divisions continued until a cyst containing seventh or eighth generation cells was formed. This then was the termination of the "division period".

The cells are spermatocytes at the end of the division period. They begin to increase in size, especially by adding to the cytoplasmic contents. This takes place during a period of suspended division activity and is the "growth period". This delay is probably a preparation for the radical changes involved in the reduction division of the "maturation period". The process of halving of chromosome numbers takes place during this period.

The period of maturation is followed by a series of transformations, largely in size and shape, in which the cells are converted into the spermatozoa (McClung, 1900).

Primordial germ cells, the primary spermatogonia, the secondary spermatogonia, and the spermatocytes are easily distinguished from each other by the relative size of the nucleus and the disposition of the chromatin material. The primordial germ cells have no recognizable nucleus, and the chromatin is scattered throughout the cell. The primary spermatogonia have a well-defined nucleus with a nuclear membrane. The secondary spermatogonial cells have a relatively small nucleus and a greater amount of cytoplasm; there is no nuclear membrane. The spermatocyte nucleus is large with a definite membrane (Sutton, 1900).

Paulmier (1899) discussed spermatogenesis in the Hemiptera; this appeared essentially the same as that in orthoptera as discussed by McClung (1900), (1941); Sutton (1900); Wilcox (1895); and Davis (1908).

Nelson (1931) did work on spermatogenesis in relation to instars with a grasshopper, Melanoplus differentialis, which basically concurred with observations of the others mentioned before.

MATERIALS AND METHODS

The cockroaches for this work were selected randomly from a culture maintained at Kansas State College. The rearing containers were metal wash tubs; water and commercial dog food biscuits were supplied in ample quantities.

The Madeira roach showed itself a satisfactory experimental animal during this study. It possessed the characteristics desired for a study of spermatogenesis: large size, ease of rearing and handling, and slow development through nymphal stages to adult. Each stadium was of ample duration to allow sufficient time and opportunity for a detailed study of the spermatogenetic activities at different intervals within each instar.

A system of measurements, using head capsule widths and distance between mandible bases, was used as a means of determining the instar of specimens from the culture tubs. Measurements were made with a micrometer in a wide field microscope. It was found that the distance between mandible bases was a more constant measurement than that of head capsule widths. Thirty-five to 45 specimens were measured in each instar.

Table 1. Distance between mandible bases of L. maderae nymphs and average duration of each stadium.

INSTAR	NUMBER OF SPECIMENS	MINIMUM (mm.)	MAXIMUM (mm.)	AVERAGE DEVIATION	AVERAGE DURATION, DAYS
1	35	0.61	0.67	0.65 ± 0.02	15.5
2	35	0.77	0.83	0.82 ± 0.02	22
3	45	0.96	1.14	1.06 ± 0.06	31.5
4	45	1.26	1.35	1.31 ± 0.05	42
5	44	1.40	1.49	1.45 ± 0.03	38
6	45	1.62	1.80	1.71 ± 0.02	57.4
7	45	2.07	2.25	2.11 ± 0.05	64
8	45	2.43	2.52	2.45 ± 0.03	71

The greatest length of the testes was measured in many cockroaches; this also contributed some information about the development of the testes during different instars. The testes were made accessible for measurement by removal of the apical five dorsal plates of the insect. An incision was made along each side of the tergal plates, from the anal port to the fifth abdominal segment. The plates thus freed were lifted away and the bulbous testes were revealed on either side of the digestive tract.

Rearing through successive instars was undertaken for confirmation of stadium size ranges established by measurements. A nymph was measured, reared into the next instar and measured again. This process was repeated until the figures arrived at by measuring had been confirmed for all eight instars. The insects were reared in pint paper ice cream containers with lids punctured for ventilation. Vials of water, stoppered by cellucotton, were placed in the containers for the water supply, and dog biscuits were used as food. The cockroaches were reared in a room with temperatures varying only from 21° to 27° C.

The testes were measured while still attached to the ejaculatory duct and then were removed for staining by severing the duct and the several tracheoles and nerve fibers which were attached. Twenty-five specimens of each instar were measured, and then observed for spermatogenetic activities.

The stains used were Gomori's chrome-alum-hematoxylin, acetocarmine and phloxine (Gatenby and Painter, 1937). Sections were not used extensively after it was found that the acetocarmine

and phloxine methods were more satisfactory and considerably faster.

The procedure in making sections follows. Testes were fixed in Baker's formal-calcium fixative or strong Flemings for six hours, impregnated with soft and then hard paraffin, and sectioned at six microns. The tissue was stained with chrome-alum-hematoxylin, and counterstained with phloxine-B, while being run through the usual alcohol baths. The nucleus was stained blue, the chromatin material very dark, and the cytoplasm red.

The phloxine smear method was very simple. The testis, in a drop of distilled water, was smeared on a slide and a pinch of phloxine powder was dissolved with the smear; this, then, was covered with a cover slip. The cytoplasm was stained light pink, the nucleus light red, and the chromatin dark red.

The acetocarmine method was nearly as simple as the phloxine method. The testis was put on a slide to which several drops of acetocarmine were added. When the testis became deep red the excess acetocarmine was blottered off. The cover slip was applied, and by gentle downward pressure the testis was crushed. The smear was then sealed with melted paraffin or vaseline and allowed to stand two or three hours to ripen and set. Results were better if the acetocarmine had been stirred with a rusty iron object. This trace of iron produced a deeper set. Again the cytoplasm stained very light pink, the nucleus red, and the chromatin very dark.

The best observations were made by use of the phase microscope with freshly dissected live material; this method was used extensively.

A record of the duration, in days, of the stadia was made; this was essential in determining how near the nymphs were to the next instar during the investigation (Table 1).

RESULTS

Testes Development

The testes of the Madeira roach were found to be paired, nearly round structures. Table 2 and Plate I show the growth rate of testes (length in mm.) in relation to instars of the nymphs; the right testis was measured in each case.

Table 2. Length of testes in nymphs of L. maderae.

INSTAR	NUMBER OF SPECIMENS	MINIMUM (mm.)	MAXIMUM (mm.)	AVERAGE DEVIATIONS
2	25	0.14	0.20	0.17 ± 0.03
3	25	0.19	0.26	0.21 ± 0.02
4	25	0.28	0.40	0.37 ± 0.02
5	25	0.40	0.74	0.63 ± 0.06
6	25	0.81	1.26	0.98 ± 0.08
7	25	1.30	1.80	1.46 ± 0.08
8	25	1.98	2.16	2.06 ± 0.03

Actual ensheathed testes were not found in the first and early second instars. The first recognizable testis was in two-week old second instar nymphs. At this time they were bud-like, oval, nearly transparent structures formed on the tips of the ejaculatory ducts. These "buds" lay one on each side of the gut in the sixth abdominal segment. Internally, the testis showed very short, slender follicles, which contained from none to very few germ cells.

The testis developed during the third instar from the oval to a more rounded shape. In this period of growth the follicles became longer and thicker as they filled with germ cells. As the follicles grew, the testes assumed a more rounded shape, but retained the transparent appearance. During this period they were found in the seventh abdominal segment and were still located beside the gut.

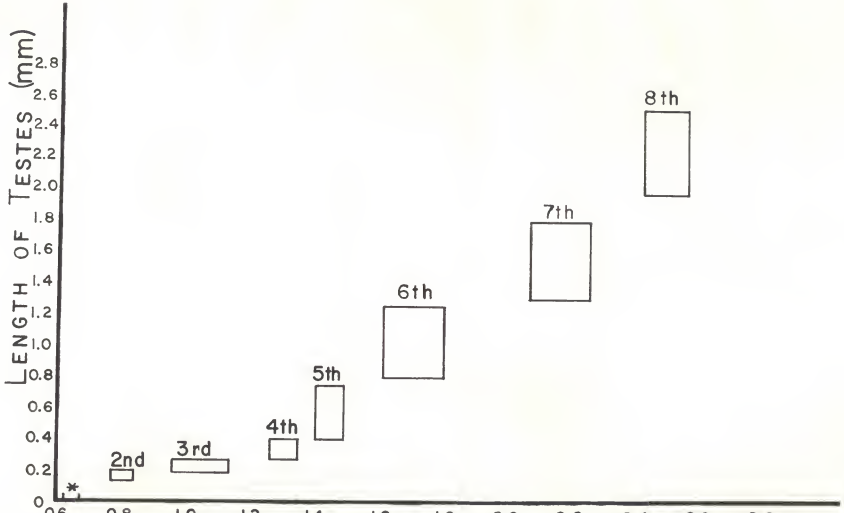
The testes of the fourth instar were still clear but had shifted laterally away from the gut and somewhat dorsally toward the posterior end of the seventh abdominal segment. Each follicle had developed a long distal finger-like projection and contained the first observed cysts of sperm cells.

Milky, opaque testes were first discovered in the fifth instar nymphs. The slender follicles of earlier instars began to fill out and become thicker in this stadium. The testes were located in the eighth abdominal segment in the older nymphs of the fifth instar.

EXPLANATION OF PLATE I

Graphic representation of Table 1 and Table 2. Minimum and maximum measurements for each instar of length of testes and distance between mandible bases.

PLATE I



*1st instar-- mandible base measurements only, no testes

The sixth instar nymphs' testes were situated in the posterior half of the eighth segment and were very near the dorsal plate. This position was maintained in the remaining stages. Fat bodies were beginning to enclose the testes. The first evidence of tailed spermatids was found in this instar.

Change in the testes of the seventh and eighth instars was largely an increase in size. This resulted chiefly from enlargement of the follicles as they became packed with developing germ cells. During these later instars the testes became fully surrounded by fat bodies.

Testes of the adult were very little different from those of seventh and eighth instar insects except in size. However, the follicles were filled with all forms of germ cells. The follicles apparently contained millions of sperm.

Terminology Used in Spermatogenesis

The literature on spermatogenesis disclosed a considerable amount of inconsistency in nomenclature. Because of minute differences in appearance of the cells, the complicated processes involved, and personal opinions, the terms used in papers have largely been the choice of each writer.

The nomenclature concerning spermatogenesis used in this paper is outlined below, and closely follows that of Sutton (1900).

Those cells attached to or in contact with the apical cell are designated as primordial germ cells and remained so until they become detached from the apical cell. (Plate II, a).

EXPLANATION OF PLATE II

Diagrams of various forms of sperm cells.

a. Distal end of follicle showing apical cell and attached primordial germ cells.
   Ap. C.--apical cell
   Fol. W.--follicular wall
   P.G.C.--primordial germ cells

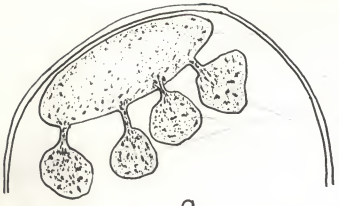
b. Primary spermatogonium

c. Secondary spermatogonium

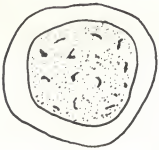
d. Four-celled cyst of secondary spermatogonia

e. Spermatocyte

PLATE II

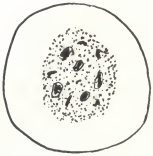


a

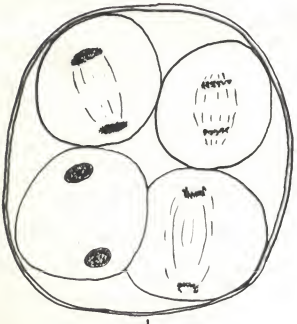


b

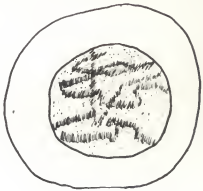
.005 mm



c



d



e

The primary spermatogonia are the earliest germ cells not attached to the apical cell and have large oval nuclei with a membrane. These cells are never found in cysts (Plate II, b).

Secondary spermatogonia are formed after repeated divisions of primary spermatogonia; these cells have no membrane outlining the nucleus and are always found in cysts (Plate II, c and d). After the last division has occurred, the chromosomes disperse and the cell enters a period of growth. Toward the end of this period the chromosomes become reorganized into a spireme, at which time the cell becomes a spermatocyte.

Spermatocytes have large nuclei with a definite nuclear membrane and a large amount of chromatin material, usually seen as a spireme (Plate II, e). These cells undergo two divisions, the first reducing tetrads to diads and the second forming spermatids from the diads.

Spermatids are those cells which have completed the two spermatocyte divisions and whose chromatin has again dispersed throughout the nucleus. Spermatids having lumps or beads of cytoplasm strung along the tail were observed in the eight instar nymphs, and are called "beaded spermatids". The various forms of spermatids are shown in Plate III.

When the spermatids have developed into smooth, long thread-like cells but are not motile they are called sperm or spermatozoa.

The age figures employed below refer to the numbers of days or weeks that a given nymph had been in that particular stadium. For instance, a two week old second instar nymph is one which had molted to that stage two weeks before examination.

Spermatogenesis

The first indication of sperm cell formation was found in second instar nymphs 15 to 18 days old. The earliest evidence was a cluster of very small primordial germ cells attached to the apical cell. Only six to eight of these cells were seen in the earlier ages, while in three-week nymphs 10 to 12 were found. These cells apparently had no nucleus; the chromatin material was seen as clumps scattered throughout the cell. There was no evidence of spermatogonia during this instar, although in the older individuals the chromatin appeared to be congregated near the center of the cell.

The level of spermatogenesis development in the young nymphs in the third instar was similar to that in the older second instar nymphs. One-week nymphs showed, for the first time, germ cells detached from the apical cell. These, which numbered about 30, were then called primary spermatogonia. All of the chromatin had moved into the center of the cell forming a nucleus which had a membrane surrounding it. In two-week nymphs the primary spermatogonia were observed in various phases of mitosis, and at the same time a new group of primordial germ cells had formed and were attached to the apical cell. Testes of three-week nymphs

contained primary spermatogonia; the most advanced of these had completed the first mitotic division while others were still in late telophase. Again, a new group of apical primordial germ cells was formed as the development of the older cells continued. Older nymphs in this instar showed a few two-celled cysts of secondary spermatogonia in the basal area of the follicles. The second group of primary spermatogonia had advanced to the level of mitotic cell division.

Fourth instar nymphs up to one week old showed only two-celled cysts plus the earlier developing stages of spermatogenesis. Eight to ten day nymphs showed four-celled cysts or first generation secondary spermatogonia (Plate II, d). These cysts were in the proximal end of the follicle with the two-celled cysts of a later group just above them. Primordial germ cell and primary spermatogonium formation and development continued as before. Nymphs three weeks old showed cysts containing up to 16 cells, which was the third generation of secondary spermatogonia. By this time the follicles contained cysts of at least three different ages in addition to the earlier stages of spermatogenesis. Nymphs in the age range of four to six weeks showed multiplication of cells in the cysts until they had undergone the final division, which was the seventh or eighth generation. It was found that a growth period occurred in the first group of encysted cells of the older nymphs in the fourth instar. Cells in a cyst were very closely synchronized in their development and mitotic activity stopped abruptly in all before the

growth period started. Different cysts, however, progressed at varying rates of speed. The formation of additional groups of primordial germ cells and primary spermatogonia continued at a rapid pace in this instar.

In early fifth instar nymphs the first three or four groups of encysted spermatogonia passed through the growth period at about the same time. A few spermatocytes were seen in three-week nymphs; such cells were easily distinguished by the presence of a spireme and a nuclear membrane, (compare c and e, Plate II). In four-week nymphs early spermatocytes had undergone the first meiotic division; this gave rise to the diads. At about the same time, the second group of secondary spermatogonia became spermatocytes. The completion of the next (second) reduction division in the more advanced cells was seen in nymphs more than four weeks within this instar. This division resulted in the formation of haploid spermatids. These cells were not actually considered spermatids until the chromatin had dispersed within the nucleus, which took place immediately after the last reduction division. At the same time (in these later days of the fifth instar) the second group of spermatocytes was undergoing the first meiotic division. The earlier processes of spermatogenesis were still taking place more distad in the follicles.

Young nymphs of the sixth instar showed approximately the same degree of spermatogenesis development as those in late fifth instar. However, the testes of one-week nymphs contained the

round spermatids characteristic of the first group and also cells of the following group undergoing the second meiotic division. A third group of spermatocytes was seen in a different phase of meiotic division. Earlier stages of spermatogenesis were observed in the distal ends of the follicles.

Spermatids went through a sequence of morphological changes during the remainder of the sixth instar. The round spermatids gradually became elongated or oval-shaped cells; some were seen with a bulge or small projection of cytoplasm, suggesting the beginning of a tail. The second group of sperm cells had become round spermatids in the second week of the sixth instar and in three-week nymphs two groups of elongated spermatids were found in most follicles. Five-week nymphs showed three or four bunches or groups of oval spermatids.

The time required for the spermatids to change from round-shaped through oval-shaped until the first evidence of a tail apparently was between four to six weeks. Older sixth instar nymphs apparently had about five to eight groups of elongated spermatids in each follicle. The chromatin material of the spermatids remained dispersed throughout the nucleus during the remainder of the sixth instar. A new group of primordial germ cells was again observed clustered around the apical cell.

In the seventh instar, it was difficult to distinguish the different groups of spermatids because of such a great number in each follicle. The oval-shaped spermatids developed through the various degrees of tailed spermatids (Plate III) into slim,

EXPLANATION OF PLATE III

Diagrams showing some of the shapes spermatids assume in their transformation into immature sperm.

a. Round spermatid

b. Elongated spermatid

c. Tailed spermatid

d. Longer tailed spermatid

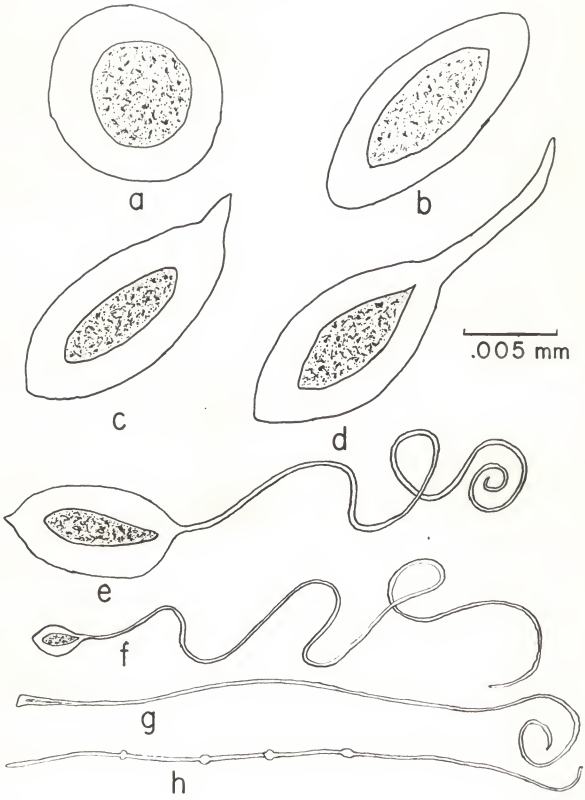
e. More advanced than (d)

f. Spermatid with very small head

g. Very late spermatid or early immature sperm

h. "Beaded spermatid"

PLATE III



non-motile, headless immature sperm. However, very few immature sperm were seen in the seventh instar. Older nymphs in this stadium showed many spermatids with very small heads and long tails. Many of these spermatids were coiled or wrapped together. All stages of spermatogenesis that occurred prior to spermatids were found in this stadium also.

In the eighth instar, peculiar forms of spermatids were seen. After the head had completely disappeared, a series of beads of cytoplasm were strung along the tail. These "beaded spermatids" were the last form of spermatids; upon losing their beads they became immature sperm. However, it did not appear that all spermatids went through the beaded condition. Testes of older eighth instar nymphs showed vast numbers of immature sperm coiled up together in a tightly tangled ball. Follicles showed all the previous stages of spermatogenesis. A few immature sperm were also seen in the vasa deferentia of some nymphs of this instar. In the nine and ten week old nymphs a few motile sperm were seen.

In adults, mature sperm were not found in great numbers in specimens younger than 10 to 15 days in most instances. Those cockroaches that were older than 15 days contained great aggregations of mature sperm. They usually were found tangled and twisted around each other in great numbers forming a spherical mass. Many others were seen in the vasa deferentia. These mature sperm were wavy or curly, and moved with a spiral-

ing motion. The testes of adults showed all stages of spermatogenesis. (Plate IV).

DISCUSSION

The correlation of (1) the growth and development of gonads, which are strictly internal organs, with (2) external cuticular landmarks, which was determined in this study, makes it possible for research workers undertaking operative procedures on cockroaches to know in advance the spermatic activity when the instar is known. It must be borne in mind, however, that two different types of growth are involved. Internal organs, such as the testes, tend to grow by means of a more or less continual but gradual increase in size. Cuticular features, such as the distance between mandibular bases used here, show an actual size increase (or growth) only at irregular intervals (i.e., immediately after ecdysses). For this reason the factor of time within a given instar is important since considerable development can occur between an early and late instar.

Plate I shows that the nymphs, at least under the rearing conditions maintained in the laboratory, were classified definitely to instar by measuring the space between mandibular bases, since, in no case did the measurements of one instar overlap those of the specimens in adjacent instars; they are quite close, however, in the fourth and fifth stages. Plate I also shows that testis dimensions were virtually continuous from one instar to the next. The exception occurred in the

EXPLANATION OF PLATE IV

Longitudinal section of a follicle of a testis of an adult Leucophaea maderae showing the zones of sperm cell formation according to instars. Diagrammatic.

a. Zone of older half of second instar

b. Zone of third instar

c. Zone of fourth instar

d. Zone of fifth instar

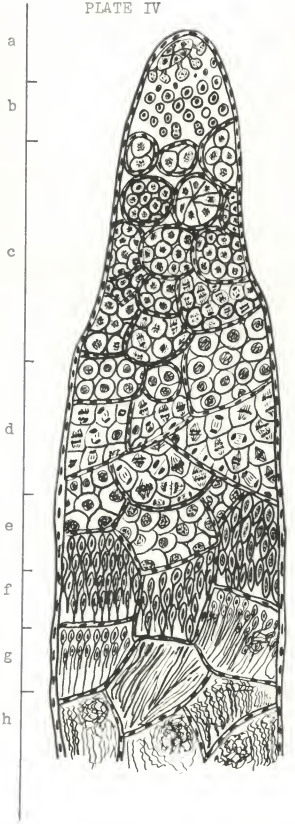
e. Zone of sixth instar

f. Zone of seventh instar

g. Zone of eighth instar

h. Zone of adult

PLATE IV



second instar, in which the upper size range overlapped the lower limits of the third.

Spermatogenetic activities were continuous once they had been initiated. The first group of primordial germ cells differentiated from the apical cell in the second instar were found as tightly wound balls of immature sperm in late eighth instar nymphs and early adults. All earlier steps of spermatogenesis were also found in the follicles of late last instar and young adult individuals.

It is possible, therefore, to predict the approximate level attained by the earliest primordial germ cells by applying the instar correlation. For example, a specimen randomly selected and found by external measurements to be in the fourth instar would have at most spermatogenetic development only to the level of encysted cells undergoing growth or specimens found by measurement to be fifth instar nymphs would have spermatids as the most advanced stage. Primordial cells formed by successive proliferations would be expected to be found in all intermediate stages of development. More precise pin-pointing of developmental levels within an instar, would, of course, entail the selection of newly molted white individuals from the culture tubs and subsequent isolated rearing. The molting date provides a base with which the spermatogenetic activities may be associated.

One other application may be made. By knowing the most advanced level of spermatogenetic development in an individual

roach, it is possible to introduce experimental procedure to a given germ cell condition and still have both testes remaining to provide for checking of results at two later time intervals.

It should be noted that the measurements and duration of instars determined here are based on specimens from one laboratory culture. Cockroaches grown under other conditions may differ to some extent. This information should, therefore, be used as a guide and should be checked for variation before being applied to specimens reared under other circumstance.

SUMMARY

The nymphs of Leucophaea maderae (F.) undergo eight instars which were determined by distance between mandibular bases. Testes were first recognizable in the middle of the second instar. Spermatogenesis commenced from mid-second instar and continued into adulthood and this process is outlined for each instar. Testes and spermatogenesis developed gradually and constantly as opposed to interrupted exoskeletal growth between instars. As a result of this study, it is possible for researchers to determine the approximate level of spermatogenesis in experimental specimens by determining the instar by external measurements.

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CORRELATION OF THE LEVEL OF SPERMATOGENESIS TO INSTARS OF *LEUCOPHAEA MADERAE* (F.) (BLATTIDAE, ORTHOPTERA)

by

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The correlation of the level of spermatogenesis to instar was the object of this investigation. This was one of the facets of a study of sexual dimorphism of cockroaches undertaken by the major professor through a Public Health Service grant.

The literature revealed very little spermatogenetic studies on cockroaches, and none was found dealing with spermatogenesis in relation to immature forms. However, the sperm formation processes of other orthopterans have been recorded and it was found that these processes were closely allied to those of the Madeira roach.

The cockroaches used were selected randomly from a culture maintained at Kansas State College in metal tubs. L. maderae, because of its relatively large size, ease of rearing and handling, and slow development through nymphal stages to adult, proved to be a very favorable experimental animal.

Measurements of various characters were recorded for the determination of instar. The distance between mandible bases proved to be the most accurate and constant factor in this determination, based upon measurements of from 35 to 45 specimens per instar. This method of determining instar was confirmed by a system of rearing specimens from one instar to the next, with measurements before and after molts.

Measurements of testes of 25 specimens per instar were recorded which gave some information as to testis development.

It was found that the testes developed gradually but constantly from mid-second instar to adult. No testes were found in nymphs of the first instar and the first half of the second instar.

Gomori's chrome-alum hematoxylin stain, Baker's formal-calcium and strong Fleming's fixatives were used in the preparation of tissue for sectioning. Acetocarmine and phloxine smear methods were also used, but the best observations were made by the use of the phase microscope with freshly dissected live material. Testes of 25 specimens per instar were observed for spermatogenetic activities by the phase microscope.

A record of the duration, in days, of the stadia was made; this was essential in determining how near the nymphs were to the next instar.

The first indication of sperm cell formation was found in mid-second instar nymphs as a cluster of primordial germ cells attached to the apical cell. These cells had no apparent nuclei.

In early third instar nymphs primary spermatogonia were first observed, and a new group of primordial germ cells had formed. The spermatogonia, now somewhat removed from the apical cell, had nuclear membranes surrounding definite nuclei. They underwent repeated divisions during the remainder of the third instar. The second group of primordial cells had become spermatogonia and a new group of germ cells had formed.

Secondary spermatogonia were first observed in very late third instar, but more often in early fourth instar nymphs. They occurred in two-celled cysts, had no nuclear membranes, and

during the greater part of the fourth instar divided rapidly forming cysts containing seventh or eighth generation spermatogonia. During the remainder of the fourth instar these cells entered the growth period, increasing in size. The cells of each cyst were in close synchronization in their development. Less advanced spermatogenetic activity continued.

In the early fifth instar, the growth period continued, and by the third week, spermatocytes were first seen. These cells had definite nuclear membranes and the chromatin had formed into a spireme. They immediately went into the divisions accomplishing the reduction of chromosome number.

Early sixth instar and very late fifth instar nymphs contained spermatids. These transformed from a round cell condition to the elongated, small-headed state during the remainder of the sixth and throughout the seventh instar.

Immature sperm, non-motile, headless, very long and thin cells first became evident in a few late seventh instar nymphs. However, the majority of nymphs did not possess immature sperm until early in the eighth instar. Some of the slower developing spermatids observed in the eighth instar had assumed a peculiar form. Series of "beads", apparently cytoplasmic, were strung along the tail of these spermatids. These beads disappeared as the spermatids developed into immature sperm. Immature sperm were also observed in the vasa deferentia of some specimens. All forms of developing sperm cells prior to immature sperm were seen in the follicles of eighth instar nymphs, also.

Mature sperm were not found in most of the cockroaches until they had been adults for about two weeks. However, mature sperm were found in some very late eighth instar nymphs. Such sperm were wavy or curly, headless, thread-like cells, moving in a spiraling motion. They were seen in great numbers, many tangled and coiled around each other forming a spherical mass containing vast numbers of them. Many mature sperm were also seen in the vasa deferentia of adults. Adult testes contained all the various degrees of sperm cell formation.

As a result of this study, it is possible for researchers to determine the approximate level of spermatogenesis in experimental specimens by determining the instar by external measurements.