THE SPERMATOGENESIS OF THE NEOTENIC SALAMANDER

EURYCEA NANA BISHOP

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THESIS

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CHAPTER I

INTRODUCTION

The large size of the chromosomes, particularly the somatic chromosomes, of the salamanders have made them popular experimental animals for cytological study. A great deal of literature exists on this subject.

The original problem of this thesis was to be a preliminary cytological comparison of the neotenic species of salamanders in Texas. The species to be studied were Eurycea nana, Eurycea neotenes, Eurycea latitans and Eurycea pterophila. The purpose of the study was threefold:

(1) to provide cytological evidence for the taxonomical division of these salamanders,

(2) to determine the worth of this experimental material for studies in population genetics,

(3) and to consider neoteny in conjunction with the two previous points.

The four species of Eurycea found on the Edwards Plateau in Texas are unique in that they have been reported as single micropopulations of extremely limited geographical distribution. E. nana is known only from one spring pool; E. latitans is known from only one cavern; and E. pterophila has been found in only one shallow stream. E. neotenes has been reported from several different localities but some

investigators (Burger, Smith and Potter, 1950) believe that more careful scrutiny will uncover localized differentiae.

Eurycea pterophila is indistinguishable from E. neotenes in external morphological features (ibid, 1950) and thus differs from E. nana and E. latitans in the same way that E. neotenes does. A number of osteological differences in individuals collected as E. neotenes however, were revealed in stained specimens and these were of sufficient note to cause the recognition of E. pterophila as another species by these authors. Clearly a cytological comparison of the four Eurycea species might help to clear up their distribution and taxonomy.

Difficulties in collecting, due in part to drought, narrowed the salamanders available to the writer to a single population, that of Eurycea nana which could be collected fairly readily from San Marcos Lake at San Marcos, Texas. In this paper an attempt is made to present a study of spermatogenesis in E. nana with the purpose in mind of providing the basis for a complete survey of the neotenic salamanders of Texas.

Eurycea nana belongs to the family Plethodontidae. An excellent summary of the literature on the cytology of this family of salamanders is given by Kezer (1948). The first classical work was done by Kingsbury (1902) at Cornell University on Desmognathus fuscus, and by Eisen (1900) whose work is a monograph on the spermatogenesis of Batrochoseps. The only modern papers on the cytology of the spermatogenetic chromosomes of Plethodontid salamanders are by Scudder (1942) and by Kezer (1948). The former paper deals with the spermatogenetic chromosomes of Desmognathus fuscus while the latter comprises a comparison of the spermatogenetic

chromosomes of Desmognathus fuscus and Plethodon glutinosus.

The chromosome number of many of the Plethodontid salamanders has been determined. From Kezer (1948): "thus, at this time 25 members of the Plethodontidae are known to possess 28 chromosomes and 2 species of this family, Hydromantas italicus and Batrachoseps attenuatus are on record as having only 24." In five forms of Eurycea, E. bislineata bislineata, E. longicauda longicauda, E. longicauda melanopleura, E. lucifuga, and E. tynerensis, counts of 28 chromosomes were made by Kezer. All counts were made from spermatocytes. Fourteen bivalents were counted at diplotene, diakinesis and metaphase I, 14 dyads at anaphase I and prophase II, and 14 chromosomes at anaphase II.
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CHAPTER II

MATERIALS AND METHODS

Eurycea nana Bishop (1941) is neotenous and, as far as is known, occurs only at its type locality in San Marcos Lake, Texas. Arising from underground springs, the cold waters of this lake form currents running out between the rocks from the edge of the lake. In Elodea and brown algae growing in these currents specimens of E. nana were collected without difficulty the year around.

The temperature of San Marcos Lake remains nearly constant both winter and summer. Collecting trips were made in June, 1951, and monthly from October, 1951 to April, 1952. A fairly uniform environment is provided for the salamanders without a noticeable seasonal change in temperature or vegetation.

It is difficult to distinguish between the sexes of E. nana in the field. The salamanders were brought into the laboratory in a container and were kept in the water in which they were collected which contained small crustacea and other food sufficient for some time. An effort was made to dissect the salamanders as soon after collection as possible, although specimens were kept alive in the laboratory during the fall months for a period of up to 54 days without additional food. It was felt that inanition and the artificial laboratory environment would affect the chromosomes.

E. nana is very susceptible to over-heating. In hot weather it was necessary to carry the collecting jar in an insulated box. In the

laboratory, the glass bowls containing the salamanders were placed in ice water which was changed every few hours to keep the temperature of the water in the bowl about 20°C. All specimens died on two occasions when heating of the water in which they were kept was caused by a sudden increase in the building temperature during cold spells.

Much time and energy were wasted at the beginning of this problem in trying to obtain good squash preparations stained with Feulgen. The aceto-orcein smear method described by Kezer (1948) was found to yield excellent slides and this method was used subsequently in this study. Optimum pressure for the smear was obtained by tapping briskly on the back of the slide with the rubber tip of a dropping bottle stopper. In some cases, one testis was submitted to the above smear method while the other was fixed in San Felice for sectioning to provide a check within the one animal as well as comparison with others. Sections were cut at 6 to 8 microns and were stained by Newton's Crystal Violet. Most of the sections were cut longitudinally but two testes were cut in cross section to complete the over-all picture.

The smear material was of most value in studying the meiotic chromosomes. Clumping of the chromosomes on the spindle and the length of the chromosomes in prophase prevented the sections from being of much value for this purpose. The spermatogenetic cycle and the internal morphology of the testis were checked from the sections.

CHAPTER III

OBSERVATIONS AND DISCUSSION

A. The Spermatogenetic Cycle

The testes of Eurycea nana are paired organs enveloped in mesentery and suspended from the dorsal body wall posterior and ventral to the kidneys. They are easily seen for they are dark in color due to the presence of chromatophores in the testicular epithelium. The intensity of color depends upon the age of the animal, the state of spermatogenesis and probably, in some cases, on the amount of diffusion of the pigment from the centers of the chromatophores into their radiating branchs. This migration of pigment has been noted by Humphrey (1921) on Desmognathus fuscus. E. nana is well spotted with pigment in all epithelia.

In Eurycea nana the writer has noticed a marked over-all color change due to "shrinkage" of the melanophores when salamanders were left in the laboratory for some time in an open vessel whose bottom was covered with fine light sand. This color change appeared to be due to a migration of pigment into the center of the pigment cells which caused an over-all effect of lightening of color. Salamanders collected from the same locale, i.e. from dark green and black algae, which were kept in a deep aquarium with vegetation from the collecting site did not lighten in color in the laboratory.

Between the two testes lie the paired Wolffian ducts. These ducts are evenly pigmented and vary in color and shape with the individual animal and the state of spermatogenesis. When they are full of sperm they

are very black in color and tend to be much convoluted and distended. In the younger males and also other males which do not have sperm in the ducts, they are light in color, thinner and less convolute. The Wolffian ducts are lined with a single layer of cuboidal epithelium (Weighert, 1945). The vasa efferentia are three or four in number and are attached for the most part anteriorly.

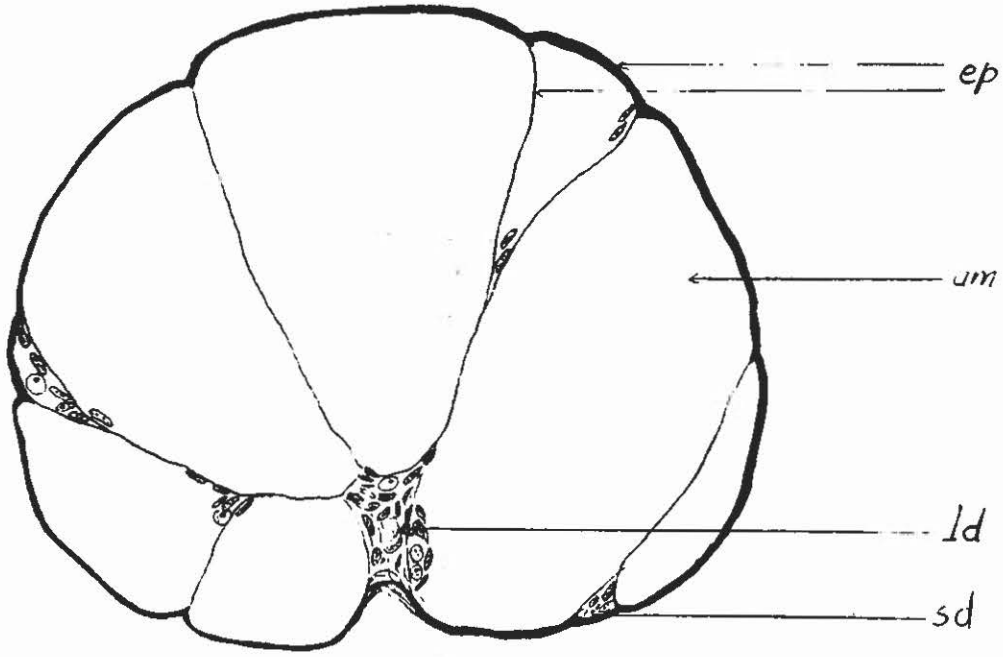
Sperm have been found in the Wolffian ducts of E. nana males from October through December and in June. Males collected in December, February and May which had 'multiple' testes also had sperm in the ducts. In some Plethodontids two breeding seasons occur during the year, a false breeding season in the fall after which no viable eggs are laid and a true breeding season which occurs usually in May or June. The breeding habits of the neotenic salamanders in Texas are not known. The presence of large numbers of sperm in the ducts in the late fall may be indicative of either true or false breeding season. It is likely that the true breeding season occurs in June since a distended condition of the ducts was noted at that time.

The salamander testis is made up of a longitudinal collecting duct and a number of lobules or ampullae separated from each other by pigmented epithelium (Diagram 1). The longitudinal duct in E. nana is peripheral for the most part. The ampullae are arranged fanwise about the duct and in each testicular division there is a network of tiny ducts which empties by one opening into the longitudinal duct. In this same region of each ampulla cluster several primary or 'residual' spermatogonia which together with the longitudinal duct make up the unchanging part of the system (Burger, 1937).

The ampullae of the salamander testis are functionally equivalent to the seminiferous tubules of other vertebrates. Unlike other vertebrates however all ampullae within the same testis do not contain germ cells in the same stage of spermatogenesis. Nearly all the cells within any one ampulla, however, are in the same stage of spermatogenesis in the salamander as are nearly all of the ampullae at any one level of the testis.

In Eurycea nana as in many urodeles, spermatogenesis passes over the whole testis in a continuous caudo-cephalic spermatogenetic wave. This term was first used by Kingsbury (1902) and is descriptive of the progression of the two meiotic divisions which begin at the posterior tip of the testis and slowly pass towards the anterior end. The time required for any one spermatogenetic stage to pass the complete length of the testis varies with the species as does also the time of initiation of the wave. Because of this unique pattern of spermatogenesis, the serial picture of the events of several months can be seen in any one testis.

The slow forward progression of the spermatogenetic wave and delayed regeneration of the lobules emptied of sperm causes a condition known as 'multiple testis' which is of common occurrence in Desmognathus fuscus and other urodeles (Humphrey, 1922). When this occurs, two or more enlargements or lobes of the testis are seen separated by intervening non-functional regions. Such multiple testes are observed only in the larger males. The smaller sexually mature males have testes of but a single lobe. In E. nana only about ten percent of the males examined possessed multiple testes. All three measured more than 50 mm. in length from tail tip to nose. Four other males examined however measuring more than 50 mm. had



Diag. 1. Cross section of testis

ep - epithelium
am - ampulla
ld - longitudinal duct
sd - secondary duct

testes of single lobe. The condition does not depend solely on large size therefore, but also on the state of spermatogenesis.

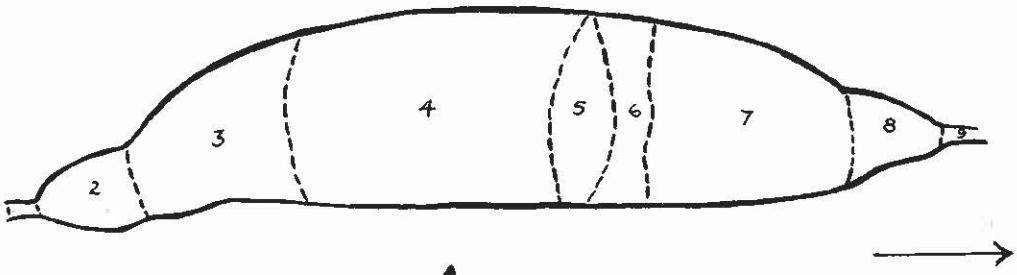
Table I gives the lengths of body and testis for all male Eurycea nana examined. The figures show the tremendous variation in size of the mature males as well as the variation of size in the testes. Generally speaking, males of length over 35 mm. were found to be mature. No correlation could be achieved between size and age. The testes of males presumably under a year old were small, single-lobed and noticeably lighter in pigment than those of older salamanders but nevertheless spermatocyte divisions were found indicating their maturity. The longest male examined was 56 mm. in length and had 'multiple testes' 6 mm. long. The longest testes found however were single lobed and were possessed by a male 51 mm. long.

Kingsbury (1902) and later Humphrey (1921, 1922) present drawings to show the order of the germ cells in the testis and their relation to the spermatogenetic wave and its resulting modification of the structure of the testis. Diagram 2 gives this information for Eurycea nana. These are longitudinal sections of the testis presented schematically. In A the approximate boundary regions of lobules containing the different cell types are shown. Beginning with the left or caudal end, region 1 consists of longitudinal duct plus primary "residual" spermatogonia; 2. empty or degenerated lobules; 3, ripe spermatozoa; 4, transforming spermatids and immature sperm; 5, spermatids; 6, divisions of first spermatocytes and resting and dividing second spermatocytes; 7, growing spermatocytes; 8, secondary spermatogonia; 9, primary spermatogonia, i.e. same as in

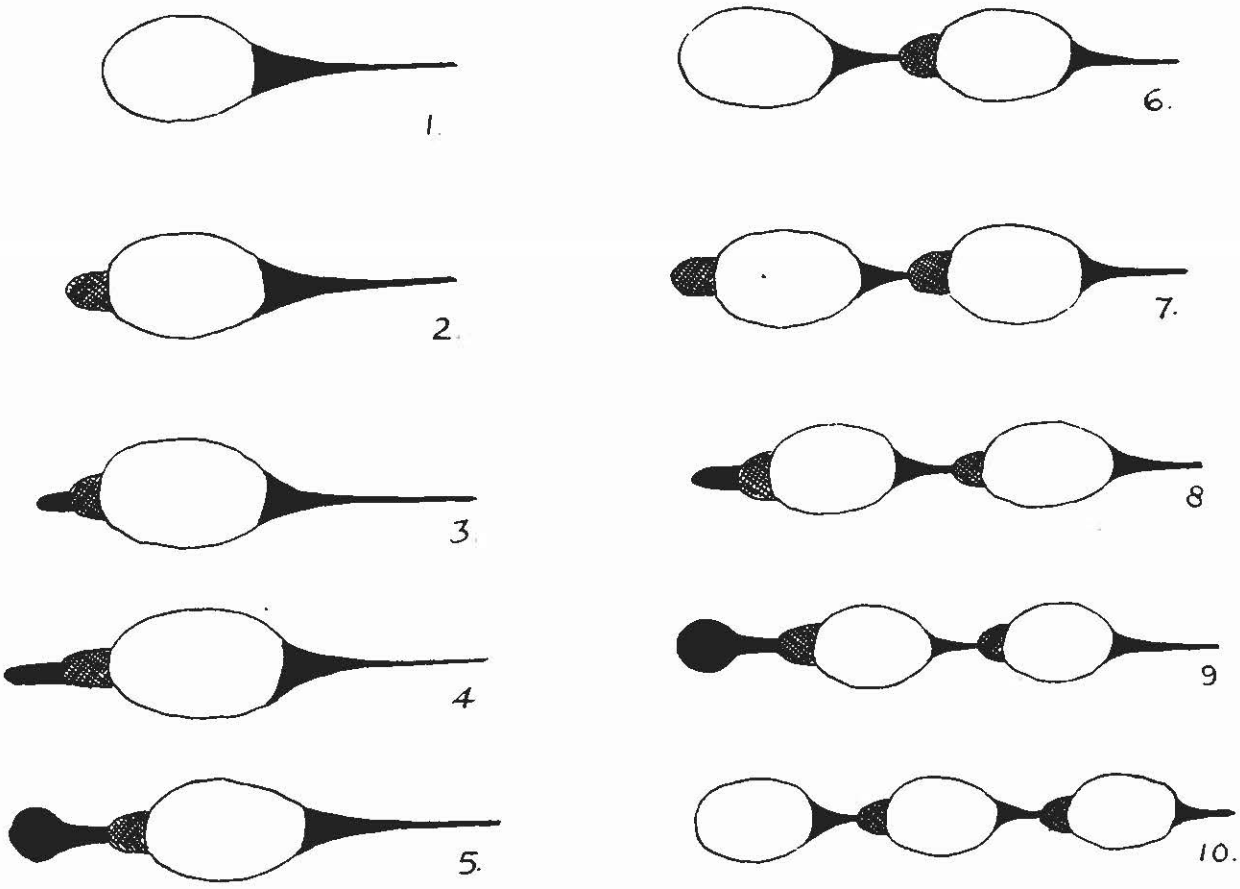
TABLE I

Date	Length of animal (mm.)	Length of testis (mm.)	Remarks
November, 1951	43	6	
December, 1951	47	6	
	42	3.5	
	52	10	CA
	45	3.5	
	45	3.5	
January, 1952	51	6.0	
	51	5	
	56	6	MT
	42	5	
	42	3	
	50	5	
	44	4	
	37	3	
	38	2.5	
	43	3	
	42	2	
	39	3	
	37	1.5	
	35	1	
	52	5	
February, 1952	52	5	
	47	4.5	
	54	5	MT
	44	4.5	
	51	8	MT
March, 1952	39.5	4	
	46	5	
	49	5	
	39	3	
	42	3.5	
April, 1952	44	5.5	MT

CA = caudal appendage
MT = multiple testis



A



B

region 1. A similar diagram is given by Kingsbury (1902) for Desmognathus fuscus in July. In Eurycea nana this distribution of successive regions has been observed in some animals collected from October through March.

In B, a series of diagrams after Humphrey (1922) gives the development of the multiple testis condition. The solid black represents regions occupied by primary or secondary spermatogonia. Unshaded areas are those occupied by later stages of the germ cells - spermatocytes I to mature spermatozoa. Crosshatched areas are occupied by degenerating lobules and interstitial cells. In three of the male Eurycea nana which on dissection were found to have multiple testes, the two testes of the individual differed in shape. In the first, the right testis was like B.9, while the left was a spermatogenetic season behind and looked like B.4. In a second animal the combination was B.10 for the right testis and B.7 for the left. A third animal showing this condition had a right organ as in B.9 while the left was as in B.8. In a fourth animal however both testes were at the same level of spermatogenesis, namely B.6. The rate of spermatogenesis seemingly varies not only between the individuals but even between the two testes of a single individual.

The shape of the caudal end of the testis as seen in B.5 and again in B.9 has caused this posterior end to be called the "caudal appendage" (Humphrey, 1925). It is caused by the appearance of a new active area due to the proliferation of primary spermatogonia in lobules emptied of sperm. Regeneration of the lobules is delayed in some species so that a thin "sterile" area is left between the preceding lobules containing maturing sperm and the regenerating lobules containing spermatogonia. The caudal appendage type of testis has been found in Eurycea nana in June, December and February.

The life history of E. nana is not known. The species appears to be entirely neotenic for no adult forms have been found. In the type locality presumably large numbers of them are consumed by fish before they attain their life expectancy. The occurrence of larger males is of low frequency in the population, the smaller ones being much more numerous.

Complete knowledge of the actual progression of spermatogenesis in E. nana is lacking because collections were not made between the months of June to October. However, by comparison with the work of other investigators on species of the Plethodontidae and by observation during the majority of months of the year, it is thought that the meiotic divisions enter the caudal end of the testis in early September. Sperm were found within the posterior third of the testis and in the Wolffian ducts from October to January with the number of sperm in the ducts being greatest in November. November is apparently a breeding season as mentioned previously. The lobules emptied of sperm in November do not regenerate until the following year. Sperm were found in the ducts of all males with multiple testes although these four specimens were collected in December, February and April. The spermatogenetic wave also progresses in multiple testes at approximately the same rate in each enlargement for this condition is simply due to more than one area of activity. No sperm were seen in the lumen of the Wolffian ducts of other males from February to April. Sperm were present in the testes of all individuals dissected from October to May however, as also were the active divisions of spermatocytes. This is not the case in Desmognathus fuscus according to Humphrey (1921) who found that sperm are not in the testes of animals collected in March, June or July, and actively dividing spermatocytes are

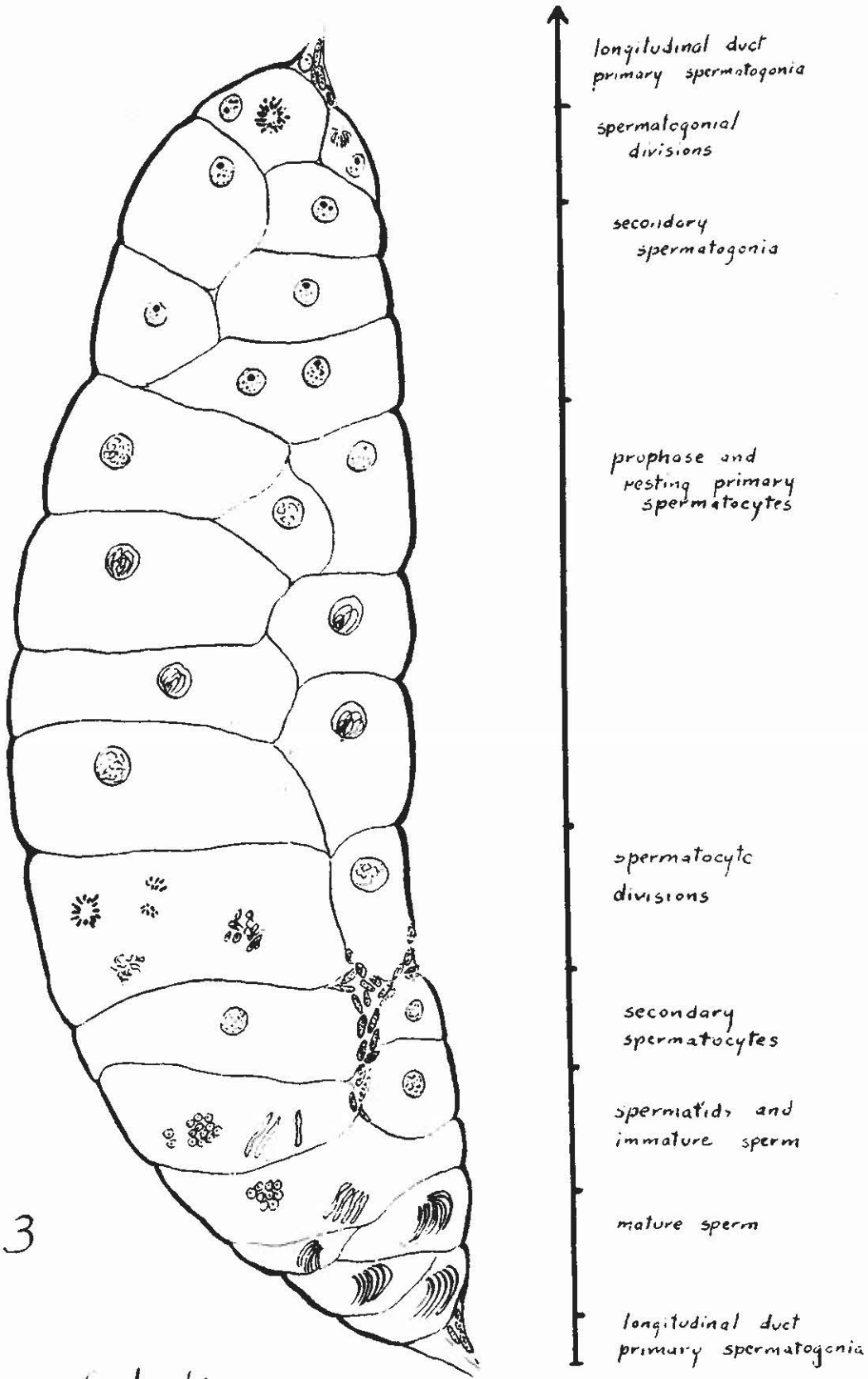
not seen from November to March.

The progression of the wave in E. nana appears to be slow and steady. The mature sperm in the testes are drained into the Wolffian ducts in the late fall and presumably discharged about the same time. Sperm which do not pass out through the duct system are pushed to the sides of the cells by the proliferating primary spermatogonia as regeneration takes place. They eventually cytolyze and disappear. From January to April the wave moves forward so that sperm and spermatids are found in the posterior half to two-thirds of the testis. During this period dividing spermatocytes are always found but division becomes more active in late March and April. Sperm have also been found in the Wolffian ducts in June and it is thought that this represents the occurrence of a true breeding season at this time. Actual observations are lacking for the summer months but it is likely that the anterior lobules are drained of sperm in June and become degenerate and that the germ cells of the posterior lobules grow and divide. In the late summer dividing spermatocytes should be found close to the caudal tip of the testis.

Diagram 3 gives the schematic aspect of a longitudinal section through a testis in February. This is the approximate appearance of the testis for the most part of the year. In each ampulla one or two cells are drawn to indicate the typical cells contained therein.

Possible Effects of Environment on Spermatogenesis:

The constancy of the water environment of Eurycea nana is remarkable. Table II sets forth the monthly temperature readings taken at San Marcos



Diag. 3

Longisection of testis

Lake 18 inches below the surface during the period June, 1951 to April, 1952. The water temperature varies only 0.5°C between the summer and winter months although the air temperature varied considerably. The pH of the water was also very constant. This information was kindly supplied by Mr. Wm. H. Brown of the Texas Game and Fish Commission in San Marcos, Texas.

The neoteny of the Eurycea species of the Edwards Plateau may be linked with the low temperature of the water in which they are found. They all are reported from cold springs, cavern or lake. In addition to the degree of coldness which would slow development, these environments are unusually constant in temperature and do not have a seasonal variation as do the species of Eurycea, Plethodon and Desmognathus which have been investigated in more northern climates. The lack of seasonal change however does not prevent the occurrence of a yearly spermatogenetic cycle. Weichert (1945) states that the testes and ducts are reawakened by cellular activity as the temperature of the air and soil approach the maximum of summer. The constancy of the environment of E. nana however does not apparently control the reproductive cycle in the male.

Temperature plays an important part in cell divisions and phenomena connected with them. Wickbom (1945) summarizes the literature dealing with chiasma frequency and temperature. Several investigators have observed the effects of abnormally high or low temperature. White (1934) and Poliakowa (1940) have found that the chiasma frequency in complete temperature series is comparatively low at a temperature normal for the organism in question but increases when the temperature is raised or lowered in a certain degree. After reviewing the literature Wickbom (1945) concludes that the dependence of the chiasma frequency on temperature is

TABLE II

Temperature and pH of San Marcos Lake, Texas

May, 1951 to April, 1952*

Date	Water Temp. °C	Air Temp. °C	pH
5-31-51	22.0	27.0	7.2
6-27-51	22.5	27.0	7.2
7-23-51	22.5	28.0	7.2
8-30-51	22.5	28.8	7.2
10-5-51	22.5	29	7.2
10-29-51	22.0	19.0	7.2
11-28-51	22.0	11.5	7.2
12-27-51	22.0	5.5	7.2
1-28-52	22.0	9.0	7.2
2-28-52	22.0	15.0	7.2
3-31-52	22.0	21.5	7.2
4-29-52	22.0	18.5	7.2

Water temperatures were recorded monthly 18 inches below the surface of San Marcos Lake.

*Unpublished data, Wm. H. Brown, Texas Game and Fish Commission, San Marcos, Texas, 1952.

probably ultimately due to changes in the viscosity of the plasma. Straub (1936) shows that the degree of terminalization is increased by low temperature probably because the latter tends to accelerate the cell division so that the chromosomes do not find time for the terminalization of chiasmata. Variations in temperature also influence the speed of the reactions which bring about the division of the chromosomes at the end of pachytene and interrupt the pairing.

The salamander, Eurycea nana, lives in spring water of low and constant temperature. The meiotic chromosomes pair distally and division is initiated from the centromere. The cold temperature might be responsible for the slowing of meiosis so that the chromosomes have time for terminalization of chiasmata for this is extensive in the late prophase I chromosomes, i.e. during diplotene to diakinesis. Low temperature also tends to inactivate the spindle and may prevent chromosome division. Bivalents have been seen to lag in attaching to the spindle. The length of the meiotic chromosomes themselves appears to be somewhat shorter than those of other members of the same family which may be due to a stronger spiralization at low temperature.

B. The Spermatogenetic Chromosomes

The Spermatogonia:

Two types of primary spermatogonial nuclei described by Scudder (1942) are also represented in smear preparations of Eurycea nana. These nuclei either take a faint stain and are lobate, or are rounded and take a normal stain. Mitoses of the primary spermatogonia are relatively infrequent, giving rise to secondary spermatogonia which are smaller and more active mitotically. Mitoses of both primary and secondary spermatogonia follow the normal sequence. Figure 11 shows the metaphase of a primary spermatogonial division showing twenty-eight chromosomes. Some of these secondary spermatogonia by growth become primary spermatocytes. The meiotic divisions in Figure 1 to 8 were drawn from spermatocyte nuclei.

The Meiotic Chromosomes:

The early primary spermatocyte:

Chromosomes have not yet been defined as discrete bodies. Scudder (1942) has found suggestions of longitudinal doubleness at this stage. He attributes this to a wider separation of the two chromatic threads at certain points and a weaker staining of the matrix so that the two threads can be seen for a short distance. He believes the "irregularly placed cross-threads are simply interchromosomal projections of the matrix". The only nuclear change in the early primary spermatocyte is an increase in size.

Leptotene to Pachytene:

The long, delicate leptotene threads first appear from the chromatic mass of the early primary spermatocyte nucleus. These are single threads and are very long and 'beaded' in appearance due to the presence of a large number of chromomeres. There do not appear to be any cross connections between the strands at this time. The ends of the chromosomes are polarized to a small area on one side of the nucleus in many cases. The leptonema threads synapse first at both ends and pairing proceeds towards the middle. The familiar "bouquet" stage is recognized from the polarized configuration of pachytene and this remains through zygotene as the synapsed strands also are looped in the same fashion. Some of the longer loops extend across the width of the nucleus while others are extremely short. As the prophase stages progress, the threads thicken and the confusion of the early prophase is resolved gradually to the more definitive forms of diplotene.

Diplotene to Diakinesis:

The chromatin becomes more smoothly staining with aceto-orcein as the chromosomes enter diplotene. As the nuclei pass through the diplonema stage, the connections between the principal threads, formerly numerous are markedly reduced in number. The majority of these connections are believed to be true chiasmata (Scudder, 1942). Figure 1 gives the configuration at diplotene. The number of chiasmata is positively correlated with the length of chromosome, i.e. the longer chromosomes possess a greater number of chiasmata than do the shorter ones. As diplotene proceeds, the chromosomes shorten and thicken. The connections

between the two threads become fewer in number and the chromosomes begin to straighten out.

The diplonema nuclei pass directly into the diakinesis stage without any more definite a distinction than increased staining density of the bivalents. The original loops become compact rings. The chiasmata terminalize as the diplotene chromosomes proceed to diakinesis. The chromosomes at diakinesis show their individual structure well in aceto-orcein smears. In Figure 2, the line of chromosomes at the top of the drawing was taken from a nucleus which showed only 13 of the 14 bivalents (plus the 14th bivalent selected from a second nucleus), but in which the chromosomes were smeared with few "overlaps" due to twisting of the bivalents in the preparation. By comparison with other figures it is assumed that the missing tetrad was due only to loss in technical handling. The other group of diakinesis chromosomes in Figure 2 was drawn from a complete nucleus to show the normal number of 14 bivalents.

At this time the spindle forms and the bivalents become attached in a single plane at right angles to the axis of the spindle. Scudder says that the diakinesis chromosomes do not all become attached simultaneously. He observed some figures in which one or two elements were completely unattached while the rest were arranged in the typical metaphase plate.

Metaphase to Telophase:

The chromosomes become more densely staining as they approach metaphase. The quadripartite nature of each chromosome becomes less distinct and, at metaphase, doubleness of the strands is shown only at the ends of the chromosomes. The spindle attachments are submedian. As the dyads move to their respective poles in metaphase I of Desmognathus fuscus

(Scudder, 1942), the space which appeared as a longitudinal split at diplonema becomes at first cross-shaped and then elongated at right angles to its original direction. The same action is seen in the metaphase I chromosomes of Eurycea nana (Figure 3). Separation of the bivalents does not proceed uniformly. Because the chromosomes vary in size, some tetrads separate earlier than others, or separate on one of their sides earlier than on the other. In Figures 3 and 4 the primary spermatocyte bivalents are shown as they begin to pull apart at early anaphase I in polar view. Figure 8 is the configuration at anaphase I, lateral view.

As the dyads move to the poles, doubleness is again clearly visible. Figure 5 shows the chromosomes in telophase just before the division of the cell. A typical resting stage does not occur upon completion of the first meiotic division.

The secondary spermatocyte division:

The arms of the dyads now rotate until X-shaped figures are seen with the chromatids held together only at the spindle attachment region (Fig. 6). These characteristically X-shaped chromosomes look much like middle prophase I chromosomes at first glance. Closer examination however shows that cross connections between the chromosomes are joinings of the two strands at the fiber attachment region as the arms spread at their distal ends, rather than the chiasmata of a four strand stage as in prophase I.

These long prophase chromosomes soon condense and become thicker, shorter and more densely staining until the second metaphase is reached. The chromosomes now are very compact and little structure can be seen

except that each dyad consists of two V-shaped or J-shaped elements (Figure 7). The individual chromosomes still retain their characteristic size. The chromosomes move apart in normal anaphase and fourteen single elements are then in position at each pole of the telophase nucleus. The cytoplasm then divides to form two spermatids.

Spermatids and Sperm:

The chromosomes of the spermatid lose their definite structure (Figure 9). The nucleus expands and the chromatic elements become diffuse and stain faintly. Most of the spermatids present in Eurycea nana males are in this diffuse stage, so it presumably lasts for some time. Gradually the nucleus elongates and the chromatin within loses all structure as the sperm develops. Figure 10 shows an immature sperm from a smear preparation. Each spermatozoa has a relatively small head which is not too sharply defined from the tail. The sperm are found in characteristic bundles or whorls in the testis and take an intense stain with Feulgen, Newton's Crystal Violet and aceto-orcein.

The Chromosome Number:

In some cases early workers made erroneous reports of chromosome numbers in the Plethodontidae due to technique. Snook and Long (1914) were the first investigators to use a smear technique and they reported the unreduced number of chromosomes in Autodax lugubris as 29 and the reduced number as 14. More recently Scudder (1942) using both sections and smears reported the same number for Desmognathus fuscus. His work was followed by Kezer (1948) who used the aceto-orcein smear method. Kezer states that of 25 members of the Plethodontidae examined, only two

possess a chromosome number differing from 2n = 28; n = 14.

In Eurycea nana, twenty-eight chromosomes were found in the primary spermatogonial metaphases. Fourteen bivalents were counted at diakinesis and in the primary spermatocyte metaphase. At anaphase these divided to give fourteen dyads migrating to each pole which are countable at the secondary spermatocyte metaphase. The chromosomal elements separate at anaphase II and fourteen chromosomes migrate to each pole. These counts were determined by careful examination of a large number of whole cells from many individuals in both sections and smear preparations.

The Size and Form of Chromosomes:

There is a tremendous variation between the sizes of chromosomes in different cells of Eurycea nana. This can be seen from the figures which for the most part are on a comparable scale of size. Other workers have classified the chromosomes of members of the family Plethodontidae according to their length. Since no actual measurements were made in this study of E. nana, and since there is some relative variation between the lengths of the chromosomal elements at different stages, this has not been attempted here. There are however three very long pairs and one very short pair of chromosomes which can be seen at all stages.

Chiasmata:

The following observations were made on chiasmata in Eurycea nana:

1. Chiasma number is roughly proportional to length, the total number of chiasmata being greatest in the longest chromosomes.
2. the number of chiasmata is greatest at diplotene and decreases regularly with the coming of diakinesis.

TxU

3. Chiasmata are reduced by the process of terminalization:

   (a) terminal chiasmata increase in number and interstitial chiasmata decrease as the prophase progresses

   (b) the distances from the ends of the chromosomes to the more distal chiasmata are inconstant both for individual elements and for a given stage of the nucleus.

These observations were also made by Scudder (1942) on the chiasmata of the chromosomes of Desmognathus fuscus. He presents a table of average numbers of chiasmata at certain stages in the primary spermatocyte prophase to support his observations.

Sex Determination:

No worker on the urodeles has reported the occurrence of morphological sex chromosomes. Nor was any pair of chromosomes in Eurycea nana distinguishable as the sex chromosomes.

The mechanism of sex determination in urodeles has not yet been proven. Support of the heterogamety of the female sex is currently in favor. Humphrey (1942) reported genetical evidence of the heterozygosity of the female in the axolotl (Amblystoma mexicanum). Triploids are known in the Urodela and they are of both sexes although the male is quite normal (Book, 1940, 1945) whereas the ovaries of the female are small and undeveloped (Fankhauser, 1938, 1940). This would indicate female heterogamety (Wickbom, 1945). The heterochromosomes in either sex are not recognizable under the microscope according to Wickbom (1945) but genetic and other observations point to female heterogamety in Urodela. He discards the evidence of Witschi (1934) for male heterozygosity in the Anura as inconclusive.

CHAPTER IV

SUMMARY

The neotenic salamander, Eurycea nana, has twenty-eight chromosomes in the primary spermatogonial metaphase. Fourteen bivalents were counted at diakinesis and in the primary spermatocyte metaphase. At anaphase these divided to give fourteen dyads which are countable at the secondary spermatocyte metaphase. The chromosomal elements separate at anaphase II and fourteen chromosomes migrate to each pole. These counts were determined by careful examination of a large number of cells from many individuals in both sections and smear preparations.

Spermatogenesis moves forward from the caudal end of the testis in a caudo-cephalic progression leaving spermatozoa in its wake. Evidence is given which indicates that the two meiotic divisions enter the caudal tip of the testis in September and that sperm are not matured in the anterior part of the testis until May or June of the following year. A discussion of the various shapes of the testis depending upon the spermatogenetic wave is given.

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FIGURES

All drawings were made at table height using an Abbe camera lucida. A 10X or 20X ocular was used and a 2 mm. N. A. 1.30 apochromatic 90X oil immersion objective.

Spermatocyte Division:

Figure 1. Middle diplotene. Aceto-orcein smear. 1500X.

2. Diakinesis. Aceto-orcein smear. 2900X.

3. Late metaphase I. Aceto-orcein smear. 1450X

4. Meta-anaphase I. Aceto-orcein smear. 1500X

5. Telophase I. Aceto-orcein smear. 1500X

6. Prophase II. Aceto-orcein smear. 1450X

7. Metaphase II, polar. Aceto-orcein smear. 1450X

8. Anaphase I. Section, Newton's crystal violet. 1450X

Figure 9. Spermatids. Section, Newton's crystal violet. 1450X

10. Sperm. Aceto-orcein smear. 1500X

11. Spermatogonial metaphase. Aceto-orcein smear. 1450X

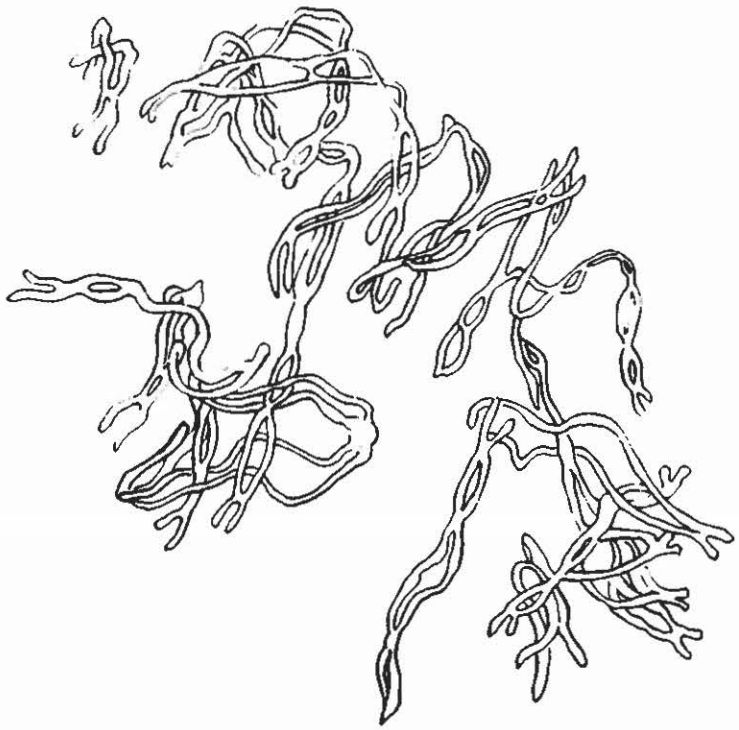
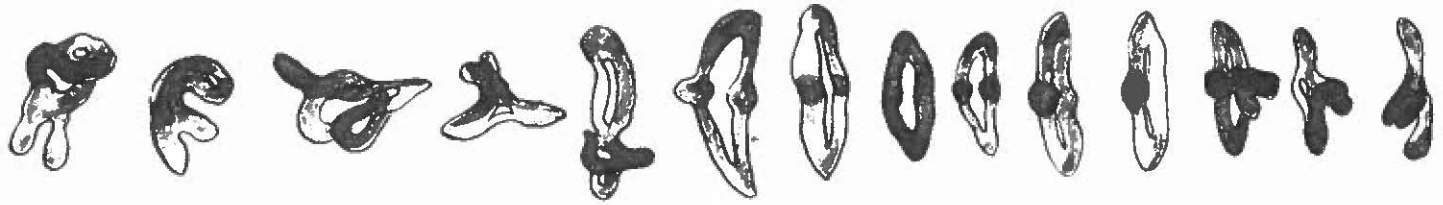


Fig. 1.



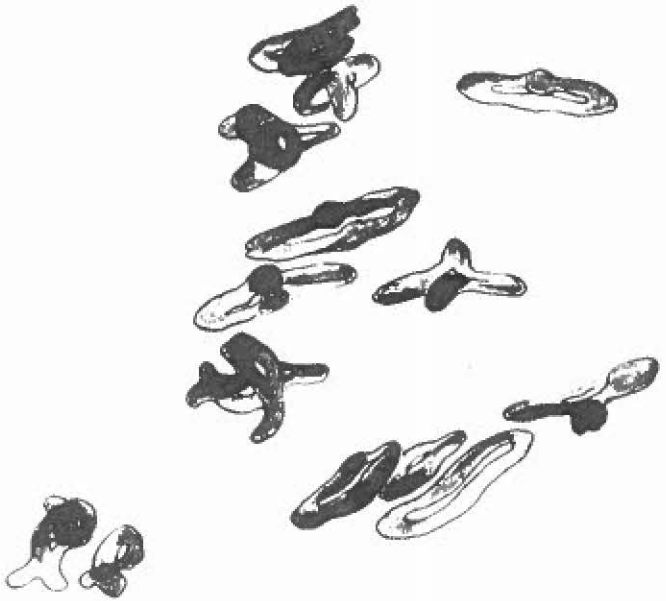


Fig. 2.

Fig. 3

Fig. 4

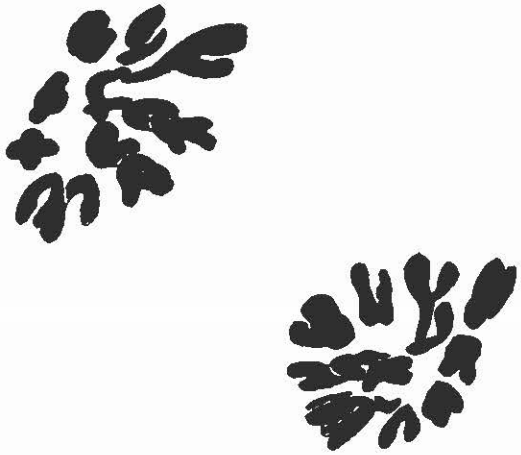


Fig. 5

Fig. 6

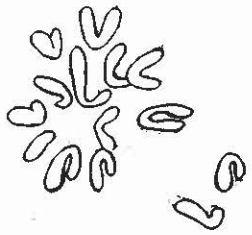


Fig. 7

Fig. 8

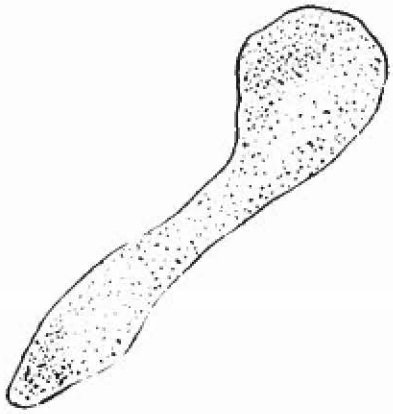


Fig. 10

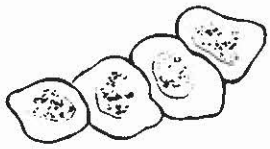


Fig. 9.

Fig. 11.

VITA

Margaret Rowlatt Mackay was born in Calgary, Alberta, Canada on March 18, 1925, the daughter of Mary Elspeth Mackay and Alexander Borthwick Mackay. She attended Earl Grey Public School and Central Collegiate Institute in Calgary, graduating from high school in June, 1943. She entered the University of Alberta, Edmonton, Alberta, in September, 1944. In April, 1948, she received a Bachelor of Science degree in Agriculture with a major in entomology from this university. Upon graduation she was employed by the Field Crop Insect Laboratory, Science Service Laboratories, Department of Agriculture at Lethbridge, Alberta. Leave of absence was granted by this Laboratory for graduate study, and in September, 1950, she entered the Graduate School at the University of Texas. From January, 1951 to June, 1952 she was the recipient of a Good Neighbor scholarship.

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