THE SPERMATOGENESIS OF
ASCARIS HABENA LINTON

by

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CONTENTS

I Introduction.

II Methods of preparation and study.

III Observations.

IV Discussion.
   A. The multiple X-complex.
   B. Numerical variation between individuals.
   C. The refractive body in the spermatozoön.

V Summary.

VI Bibliography.

VII Plates.
I. INTRODUCTION

In recent years, a great deal of attention has been directed by cytologists to the detailed study of the chromosomes in many species of animals. The question is often asked: Is the vast amount of labor expended in collecting all this infinite detail justified? The fact that chromosomes have been shown to be closely correlated with the phenomena of heredity gives them an importance beyond a mere interest in their peculiar behavior. McClung (197) says: "It is the belief that the substances of the chromosomes are specific materials which are intimately concerned with the development of a multitude of dissimilar cells from a single cell that renders a knowledge of the finest details of their structure and behavior of the utmost importance." Studies of chromosomes in lower forms have brought to light facts which assist in interpreting more complex phenomena in other animals. It is from this mass of details that significant facts have been sifted out which have led to the formulation of new theories; and in turn, the various theories of chromosome behavior are supported or disproved by additional evidence. It is with the intention of adding to the catalogue of facts that this paper is presented.

In the development of spermatozoa, there are first differentiated in the embryo the primordial germ cells. These divide and subdivide until a large number of cells, known as spermatogonia, are formed. Then the cells begin to increase in size and following this growth period, there
are two maturation divisions during which the chromatin content becomes reduced one half. The cells produced by these divisions become immediately the functional germ cells, or spermatozoa. It is the behavior of the chromosomes of Ascaris habena during the maturation divisions that is discussed in the following pages.

This study was undertaken at the suggestion of Dr. George Lefevre, and has been pursued under his direction. My thanks are due him for kindly aid and criticism as the work progressed.

II. METHODS OF PREPARATION AND STUDY

Ascaris habena Linton is a nematode worm parasitic in the digestive tract of the toad fish, Opsanus tau. The worms were quite abundant, and out of six toad fish killed, only one failed to show the parasites. There were from three to a dozen worms in each fish. The material for this work was collected at Woods Hole, Massachusetts, during the summer of 1917.

The worms were dissected in body fluid under a binocular microscope and the long, thread-like testes were placed immediately in fixing fluid. Bouin's fluid, Carnoy's fluid, strong Flemming, and an alcohol-acetic mixture were used for fixing. The testes were later sectioned and stained, for the most part, in Heidenhain's iron haematoxylin. Bouin's fluid proved to be the most satisfactory of the fixatives. The material fixed in Flemming's fluid became so broken up in trans-
portation from Massachusetts to Missouri that it was of little value.

The slides were examined under a 2 mm apochromatic oil immersion objective and a number 8 or a number 12 compensating ocular. The drawings were all made with the camera lucida, at table level, using the number 12 ocular. These drawings were corrected with the number 8 ocular, then enlarged two and one half times. The finished outlines were again compared with the sections and finally corrected. As reproduced in the photographs, the figures show a magnification of 4321 diameters.

In fixing the material, the individuals were not carefully isolated, but in some cases gonads from several specimens were preserved in the same vial to save space. In the sectioned material some differences were observed between different slides and an effort to correlate these differences with different individuals was only partially successful. However, the large pieces of testes were kept separate during dehydration, and serially sectioned. In all slides from any part of a single piece of gonad the cells were similar, and in no instance did more than one of the types of cells occur in a single piece of material. It is thought probable, therefore, that, in cases where two different pieces of gonad showed two different types of cells, the pieces came from two different individuals.
III. OBSERVATIONS

There were three types of cells observed which I shall call A, B, and C. Of type A, three large separate pieces of gonads, which may be three individuals, were observed; of type B, two; and of type C, three. Externally, there were no noticeable differences in the worms; the only apparent variation was in the chromosome numbers.

No spermatogonia were observed. The prophases of the first maturation division were the earliest stages found, and here the chromosomes were scattered about the nucleus as large, clear cut, dumb bell shaped bodies. As it was not possible to obtain an accurate count of these bodies in sectioned material, a study of this stage in smear preparations would be of value. The centrosome was seen at first on one side of the nucleus, but later it divided and one part moved to the opposite side (figures 1 and 2). During the formation of the spindle, with the subsequent orientation of the chromosomes, the nuclear membrane did not dissolve. Figure 3 shows this membrane still intact except at the points immediately opposite the centrosomes.

Polar views of the equatorial plates in the late prophase and metaphase were carefully studied. Type A showed sixteen chromosomes, type B seventeen, and type C eighteen. In type A, the most common arrangement showed ten chromosomes in an outer ring, five in a smaller ring inside, with one chromosome in the center (figure 4). Type B is represented by figures 9 and 10. In figure 10 there are shown fourteen
chromosomes scattered about the periphery of the plate, and in the center are three intimately associated chromosomes which may possibly be the X-chromosomes. Type C showed two conspicuously large chromosomes. Figures 16 and 17 are polar views of metaphase plates of this type drawn from different slides. The two largest chromosomes differ in shape which may be explained by a possible difference in fixation.

Some of these chromosomes were clearly bivalent in character, while others were so condensed as to fail to show any division. In accordance with the known facts of spermatogenesis, we should expect fourteen to be bivalent, and the remainder univalent. Only a study of the spermatogonial stages can make clear this part of the work.

The anaphase stages were studied both from side and polar views. In quite early stages, there was seen between the main groups of separating chromosomes, a mass of chromosomes which appeared to be lagging. From a polar view in one particularly favorable cell of type A, there were counted fourteen chromosomes at the highest level; a trifle lower were two large chromosomes close together which seemed to be attached to the upper group of fourteen by a deeply staining mass; while at a still lower level, was another group of fourteen chromosomes. The arrangement of the chromosomes in the two groups of fourteen was exactly similar, homologous chromosomes appearing opposite one another. Figure 6 (a) shows this cell with the chromosomes in place, while (b) was drawn with the groups somewhat separated. Another cell
from type A is shown in figure 5. This is a side view and the polar groups are not shown in their entirety. The lagging group of chromosomes is the thing of particular interest, and must undoubtedly be the X-complex which here consists of two distinct chromosomes.

Turning now to the same stage in type B, it was found that in side view there were three chromosomes in the X-group (figures 11 and 12). A polar view is shown in figure 13 with two groups of fourteen autosomes, and between them the tripartite X-complex. In the actual cell, the upper autosome group was superimposed upon the lower one; therefore, for the sake of clearness, the drawing was made with the two groups separated.

In type C, the X-complex consisted of four parts. Figure 18 illustrates a late anaphase from side view.

A great many polar views of the separating groups of autosomes in all types of individuals were examined. They all showed fourteen autosomes arranged in the form of a double circle. Figures 19 and 20 are typical.

The nuclear membrane, so conspicuous in the metaphase plates, still persisted well into the anaphase. The condition shown in figure 12 was not at all uncommon. As the cells passed from the first maturation division directly into the second without an interkinesis and therefore with no nuclear membrane, one of the most conspicuous distinctions between the metaphase plates of the first spermatocytes and those of the second was the absence of the nucle-
ar membrane in the latter. Compare figures 4 and 7.

Since the X-chromosome complex passed undivided to one pole in the first maturation division, we should expect to find two kinds of second spermatocytes in each type of individual, while of course the differences between the types will persist. Exactly this was found to be the case. In type A the second spermatocytes contained fourteen and sixteen chromosomes; in B they contained fourteen and seventeen; while in C there were fourteen and eighteen. A large number of accurate counts of chromosomes in flat equatorial plates were made, the results of which are indicated in the following table.

Table of second spermatocyte chromosome counts.

<table>
<thead>
<tr>
<th>Number of chromosomes in second spermatocytes</th>
<th>Number of cells</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type A: Type B: Type C</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>200: 75: 45</td>
<td>320</td>
</tr>
<tr>
<td>16</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>73</td>
<td>296</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>55</td>
</tr>
</tbody>
</table>

The figures represent the number of cells counted at random, which contained the various numbers of chromosomes. It will be noted that in each type the two kinds of second spermatocytes were found in approximately equal numbers. This is to be expected as a result of the unequal distri-
bution of chromosomes in the first spermatocyte division, where one of the poles received the undivided X-group and the other did not.

Figures 7, 8(a), and 8(b) illustrate the typical second spermatocytes of type A; figures 14 and 15, of type B; and figures 21, 22, 23, and 24, of type C. The equatorial plates shown in figures 21 and 22 are from the same individual as the first spermatocyte of figure 16; likewise, the plates shown in figures 23, 24, and 17 are from a single individual. In this last individual, it was clear that the two long, curved chromosomes belonged to the X-group, as they appeared in only one half of the second spermatocytes. The small size of the chromosomes in figures 23 and 24 is due to the extreme extraction of the stain. Those second spermatocytes which contained fourteen chromosomes were quite similar in all types.

Side views of the metaphases of the second division had the appearance of figure 25, where the absence of the nuclear membrane made them readily distinguishable from the same stage of the preceding division (fig. 3).

In the anaphase stages, the centrosphere became very large and stained deeply. Polar counts of the late anaphase could not be obtained as the chromosomes were closely massed in my material. In side view the separating groups were of practically equal mass and there were no lagging nor precociously dividing chromosomes; it may therefore be assumed that this is probably an equational division. Figures 26
and 27 show side views of this anaphase.

The spermatids and spermatozoa were rounded bodies having the chromatin condensed in a small area on one side of a peculiar refractive body. Figure 28 is a spermatid from the testis, while figure 29 is a spermatozoön found in the oviduct.

DISCUSSION

A. The multiple X-complex

A comparison of the peculiarities of chromosome behavior in Ascaris habena with those of other species of animals reported in recent literature throws some light upon the significance of certain phases in this form.

An X-complex of several chromosomes has been described in a number of other animals. The typical condition of the X is a single chromosome which may or may not be mated with a Y. This type of X is illustrated in the nematode, Heterakis, where the single X is not mated with a Y (Gulick, '11).

The genus Ascaris offers an interesting series of gradually increasing complexity. In Ascaris felis, there is only one X-chromosome, twice the size of its mate, the Y, which behaves in a typical manner, passing undivided to one pole in the first maturation division. The behavior of the multiple X in Ascaris habena has been described at length in the preceding pages. In Ascaris lumbricoides, Edwards ('10) reported an X-complex of five elements unmated with a Y, which passes as a unit to one pole in the first maturation division. There are thirty-eight autosomes in this animal,
so that the second spermatocytes contain nineteen and twenty-four chromosomes. The second maturation division is equational.

Ascaris canis has an X of six parts which is also unmated; the first division is reductional and the second, equational. In Ascaris incurva, there are three bivalent autosomes; while the X-group, the largest yet observed, is composed of eight elements mated with a single Y. The first division is reductional, producing secondary spermatocytes of fourteen and twenty-one chromosomes respectively, and the second division is equational.

Among the Hemiptera, there are a number of cases of unusual sex chromosomes. Payne studied many species of the family Reduviidae, and the following list includes the forms which he studied and their combinations of sex chromosomes.

- Acholla multiispinosa——5X—Y
- Conorhinus sanguisugus——2X—Y
- Fitchia spinosula———3X—Y
- Phriontis modesta———4X—Y
- Prionidus cristatus———3X—Y
- Pselliodes cinctus———3X—Y
- Roconnata annulicornis——2X—Y
- Sinea (4 species)———3X—Y
- Sinea releyi———5X—Y

In the cases of Acholla, Prionidus, and Pselliodes, the mass of the Y-chromosome is equal to, or in excess of, the combined masses of the X-elements. In other cases the
Y is comparatively smaller.

Many other Hemiptera may be cited showing similar peculiarities. Wilson ('09) reported a double X condition in Syromastes. Payne ('08) found a quadripartite X-element in Gelastocoris (Galgulus) which was mated with a Y. The second division was reductional and produced spermatids containing sixteen and nineteen chromosomes respectively. In Lygaeus (Wilson, '12), the condition is similar to that in Ascaris felis where the single X is twice the size of its mate. In Thyanta, there are two parts to the X; one species shows these mated with a Y, while another species lacks the Y. Morgan ('09), in Phylloxera fallax and Phylloxera caryaeaulis, found an X of two parts which passed as a unit to one pole in the first spermatocyte division. Payne ('13) found in Gryllotalpa an unequal pair of idiochromosomes. In addition to this, there is an odd chromosome which always passes to the same pole as the larger idiochromosome in the reductional division. The large idiochromosome and the odd chromosome are in no way connected, but they invariably behave alike, and, therefore, have a distribution with relation to sex.

The following diagram graphically illustrates the above combinations of X-chromosomes. The figures show side views of the equatorial plates with the X-elements on one side; and the Y, when present, on the other side, stippled to distinguish it from the X. Some of the data for this figure were obtained from a figure by Wilson ('11, p. 89).
Diagram of sex chromosomes

- Heterakis
- Thyanta "A"
- Syromastes Homo
- Ascaris habena
- Ascaris lumbricoides Ascaris canis
- Ascaris felis Thyanta "B" Lygaeus
- Roconnata Fitchia
- Conorhinus
- Sinea (3 sp.) Prionidus Pseiliodes
- Gelastocoris Sinea diadema
- Acholla Sinea releyi Ascaris incurva
The origin of the single unmated X-chromosome and of the multiple X-chromosomes has been the subject of considerable discussion. Wilson ('11) discussed the possibility of an evolution from a condition like that in Ascaris megalocephala, where one end of one of the long chromosomes is probably the X, to a condition where there is a single unattached X which may itself divide to form the multiple X-group found in many forms. To quote from Goodrich, ('16,p. 71):

"The genus Ascaris, as pointed out by Wilson ('11) in the case of Ascaris megalocephala, gives some basis for the suggestion of Stevens ('09) that an unmated X may form by release of X-chromatin from a Y-XX bivalent thus leaving the Y-Y portion to function as a bivalent and the X-chromatin as a univalent chromosome. In Ascaris felis the group is an unequal tetrad, the larger component not being visibly compound; in Ascaris incurva the X-element is clearly compound but is still united to the Y-Y portion; Ascaris megalocephala shows an X-element sometimes united and again separate from the Y-Y (?) chromosomes which are in this case recognized as autosomes; while in Ascaris lumbricoideae it may be that the separation of the compound X from the Y-Y pair has taken place. In this respect the chromosome complex of the genus Ascaris most closely resembles that of Orthoptera such as Hesperotettix, Anabrus (McClung '05) or Leptnia (de Sinety '01) in the association of the X with an autosome or a Y-Y group."

As to the behavior of the large X-group, there is no evidence in Ascaris habena concerning stages other than those
of the maturation divisions. In Ascaris incurva, the X-group does not act as a unit except during the maturation divisions, and Goodrich suggested that this might serve as a mechanism for the shuffling of sex-linked characteristics to a much greater extent than has been observed in the crossing-over phenomena in Drosophila.

B. Numerical variation between individuals

One of the most striking facts, in connection with the study of Ascaris habena, is the numerical variation in chromosome numbers in different individuals. Formerly, it was thought that all the individuals of a given species possessed the same number of chromosomes.

McClung ('17) recently published some interesting results on multiple chromosomes in Hesperotettix viridis, in which he described some six different kinds of individuals. In this species, some of the chromosomes fail at times to unite into tetrads, or some combine with already formed tetrads to form hexads or octads. In any one individual the condition is always constant. In several other species, H. speciosus, H. pratensis, and Mermiria bivitata, the accessory chromosome becomes united to one of the euchromosomes or autosomes. In these last species, however, the condition is always constant and shows no individual variation.

In this connection, the case of Ascaris megalcephala is interesting. Here the end of one of the long chromosomes is probably the X, although in some cells of this species,
the X is not joined to the longer autosome but is found free. Cells containing the small free chromosome are found side by side in the same animal with cells lacking it. The long autosomes and the small X may be considered multiple chromosomes when compared with the small chromosomes found in the cells of the soma; for example, Kautzsch and Geinitz, as quoted by Goodrich, showed that the X in the maturation divisions of the germ cells is represented by eight or nine smaller chromosomes in the cells of the soma.

There are two varieties of this species, Ascaris megalococephala bivalens and Ascaris megalococephala univalens, containing respectively four and two chromosomes. Miss Boring ('10) obtained material for study from twelve different horses, securing eighteen worms in all; of these, twelve were bivalens and six univalens. In five cases, the two varieties were found in the same host, and Miss Boring suggested that it might not be impossible that all the worms in a single host had a common heritage.

Payne ('14) reported striking conditions in the European earwig, Forficula. There are irregularities here in chromosomal distribution which may vary within a single individual. In cases where there is a spermatogonial count of twenty-four, the first spermatocytes show twelve, thirteen, or fourteen chromosomes. In the case of the twelve-group, all are bivalent; in the thirteen-group, eleven are bivalent and two single; and in the fourteen-group, ten are bivalent and four single. The second maturation division shows the
single chromosomes behaving in a very irregular manner, some-
times all passing to one pole, sometimes dividing more or
less equally, and sometimes remaining on the spindle in a
very much elongated condition. This results in spermatids
containing from ten to fourteen chromosomes. Payne explained
these peculiar occurrences by assuming that some of the
spermatogonial chromosomes failed to pair in synapsis, and
therefore remained univalent. He drew no conclusion from the
irregular behavior of these univalent chromosomes in the
second division.

Cases of supernumerary chromosomes might be mentioned
here. In Metapodius (Hemiptera), Wilson ('09) found that
there occur occasionally small supernumerary chromosomes
varying in number from one to six. These probably originated
by the failure of some of the small chromosomes to divide
normally at some time. If both members of a pair should pass
to one pole, the resulting spermatozoa would have an unusual
number of chromosomes. In considering the probable small
number of such abnormal divisions, and the fact that only
a few of all the spermatozoa produced ever fertilize eggs,
it can be seen that only a few individuals will show the
supernumerary chromosomes.

In Diabrotica soror and Diabrotica 12-punctata, Miss
Stevens ('08) described variations in the chromosome numbers.
The differences are due to several small supernumerary
chromosomes which appear in the spermatogonial plates and
throughout maturation. They divide in only one of the
maturation divisions (not always the same one) and give rise to several kinds of spermatozoa. These supernumerary chromosomes vary in number from one to four, but the number is constant in any given individual. Miss Boring suggested that these differences might be due to hybridism.

The foregoing variations serve to show that numerical constancy is not a necessity in a species. In the case of Ascaris habena, it is difficult to interpret the numerical variation. It seems to be limited to the X-chromosomes, for the autosomes behave in a normal and consistent manner in all cases. From the point of view of mass, the two X-chromosomes of type A do not seem to be large enough to include the chromatin of the quadripartite X-group of type C, so that an explanation on the basis of multiple chromosomes seems unlikely. There is of course the possibility that there is more than one species under consideration. However, Linton ('99) reported no more than one species of nematode in Opsanus tau, but he examined only two fish and obtained eight parasites.

Wilson ('11) pointed out four possible ways by which chromosome numbers may change from species to species, and the same may also apply to changes from individual to individual.

1. By gradual fusion of separate chromosomes to form multiple chromosomes,—or the reverse, separation of chromosomes.
(2) By gradual disappearance of individual chromosomes, as is suspected by some investigators in the cases of the Y and M chromosomes.

(3) By sudden mutations.

(4) By abnormalities in mitosis where two allelomorphs pass to one pole instead of dividing.

C. The refractive body of the spermatozoön

The spermatids in Ascaris habena form the typical refractive body found in so many species of Ascaris. Wildman ('12) studied this refractive body in Ascaris megaloecephala and found that it arises from karyochromatin given off by the nucleus. These bits of chromatin secrete yolk within themselves and gradually fuse to make the large refractive body. The function of this body is purely nutritive and it is sometimes entirely consumed by the spermatozoön to obtain energy to reach the ovum. In figure 29, the refractive body appears to be undergoing degeneration. The spermatozoön has completed a large part of the journey through the oviduct, and this degeneration is in line with Wildman's hypothesis that the material of the refractive body is used for food.

SUMMARY

1. Three types of individuals are found containing sixteen, seventeen, and eighteen chromosomes, respectively, in the first spermatocytes. Of these, probably fourteen
are bivalent and the remainder univalent.

2. The three types of second spermatocytes show fourteen and sixteen, fourteen and seventeen, and fourteen and eighteen univalent chromosomes, respectively.

3. The autosome count is the same in all cases, namely, fourteen.

4. The X-complex in the three types consists of two, three, and four chromosomes, respectively.

5. The first maturation division is reductio nal, the second equational.

6. The nuclear membrane persists in the first maturation division until the late anaphase.

7. No interkinesis occurs between the first and second maturation divisions, and, therefore, no nuclear membrane is seen in the second division.

8. The spermatozoa are typical of the genus Ascaris.
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Description of figures

All figures were drawn with a Zeiss 2 mm apochromatic objective, a number 12 compensating ocular, and projected with the camera lucida to table level. The figures as here photographed give a magnification of 4321 diameters. All photographs were made by Professor G. S. Dodds.

PLATE I

Explanation of figures

1. Early prophase of first maturation division, showing undivided centrosome outside nuclear membrane.
2. Side view of prophase of first maturation division showing formation of spindle.
4. Polar view of metaphase of first maturation division in type A, showing sixteen chromosomes.
5. Side view of anaphase of first maturation division in type A showing two X-chromosomes.
6(a). Polar view of anaphase of first maturation division showing two X-chromosomes between two groups of fourteen autosomes.
   (b). Same cell as (a) drawn with chromosomes separated.
7. Polar view of metaphase of second maturation division showing fourteen chromosomes. Type A.
8(a). Polar view of metaphase of second maturation
division showing fourteen chromosomes. Type A.

8(b). Another cell in same stage as figure 8(a).
PLATE II

Explanation of figures

9. Polar view of metaphase of first maturation division in type B showing seventeen chromosomes.

10. Same stage as figure 9. The three chromosomes in the center may be the X-chromosomes.

11. Side view of anaphase of first maturation division in type B showing three X-chromosomes.

12. Another cell similar to that shown in figure 11.

13. Polar view of anaphase of first maturation division in type B showing three X-chromosomes between two groups of fourteen autosomes. Separate drawings were made of the chromosomes at different levels.

14. Polar view of metaphase of second maturation division showing fourteen chromosomes. Type B.

15. Polar view of metaphase of second maturation division showing seventeen chromosomes. Type B.
SPERMATOCYESIS OF ASCARIS HABENA

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PLATE II

9  10

11  12  13

14  15

-38-
PLATE III

Explanation of figures

16. Polar view of metaphase of first maturation division in type C showing eighteen chromosomes.

17. Another cell in same stage as figure 16.

18. Side view of anaphase of first maturation division showing four X-chromosomes. Type C.

19. Polar view of autosomes at one pole of anaphase of first maturation division. Common to all types.

20. Same as figure 19.

21. Polar view of metaphase of second maturation division showing fourteen chromosomes. Type C.

22. Polar view of metaphase of second maturation division showing eighteen chromosomes. Type C.

23. Same stage as figure 21, greatly extracted.

24. Same stage as figure 22, greatly extracted.
SPERMATOCYTES OF ASCARIS HABENA

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PLATE III
PLATE IV

Explanation of figures

25. Side view of metaphase of second maturation division.


27. Side view of late anaphase of second maturation division.

28. Spermatid from testis.

29. Spermatozoön from oviduct.
SPERMATogenesis of Ascaris habena

H. Hibbard

PLATE IV
The dissertation—The Spermatogenesis of Ascaris latum Linnaeus—presented by Miss Hope Hildyard for the degree of Master of Arts is approved.

George Lefever

May 13, 1918.