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TRANSACTIONS
OF THE
WISCONSIN ACADEMY
OF
SCIENCES, ARTS AND LETTERS

VOL. XVI, PART I. No.

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MADISON
DEMOCRAT PRINTING COMPANY, STATE PRINTER.
APRIL, 1908.

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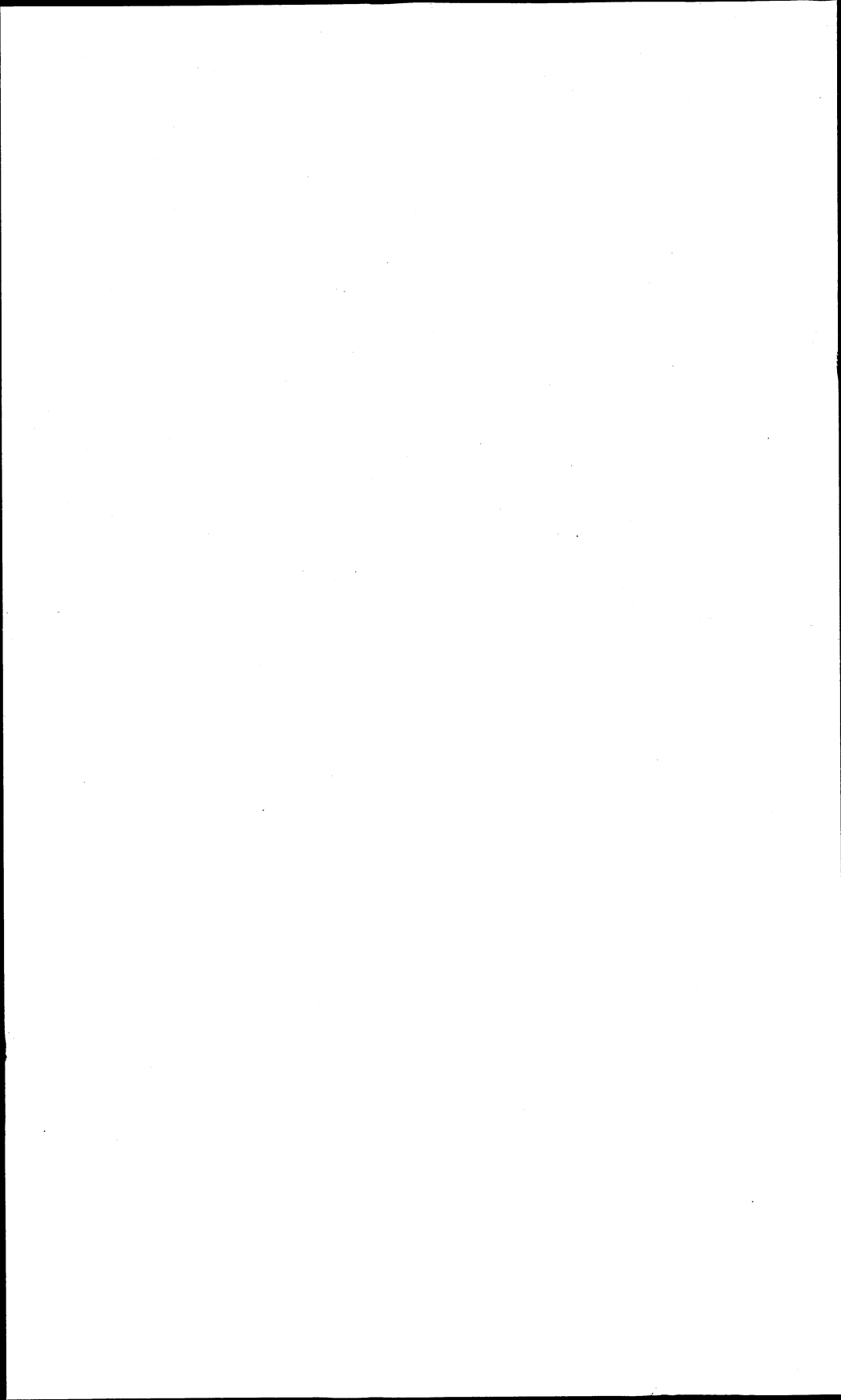
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Beginning with Volume XVI, the Transactions of the Wisconsin Academy of Sciences, Arts and Letters will be issued in smaller and more frequent numbers than heretofore. A volume will consist of five or six or more parts of approximately one hundred pages each, issued at intervals during two years.

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A SUMMER RESTING STAGE IN THE DEVELOPMENT OF CYCLOPS BICUSPIDATUS CLAUS.

E. A. BIRGE AND C. JUDAY.

[Biological Notes from the Laboratory of the Wisconsin Geological and Natural History Survey. I.]

In examining some mud from the bottom of Lake Mendota, brought into the laboratory of the Survey in the fall of 1905, a small oval body was found, whose exterior was composed of particles of the mud and in whose interior we expected to find a winter egg or similar resting stage of some animal. Dissection showed, however, that the cocoon contained a well developed, though sexually immature *Cyclops*, and further study showed that the mud held a great number of these cocoons, from which the *Cyclops* soon hatched out in the laboratory. During the season of 1906, the history of this peculiar resting stage was investigated as closely as circumstances permitted, with the results which are given in the following paper.

The annual development of the genus *Cyclops* in Lake Mendota was described in a paper published in 1897, and is in brief as follows (Birge, '97, pp. 326-335): In April of each year the spring development of *Cyclops* begins and the numbers rise rapidly to a maximum. In 1895, the maximum was during the month of May, when the average number per square meter of surface was over 900,000. In 1896, the maximum extended from the middle of April to the middle of May, with an average of over 1,400,000 per square meter of surface. This enormous number of animals is chiefly composed of immature individuals, very few of them reaching sexual maturity. At the period of maximum development they are distributed pretty

uniformly throughout the entire depth of the water, which reaches about 22 meters. As the period of maximum development passes, the crustacea aggregate in the lower water, declining in number in the upper and especially in the middle strata. In the latter part of May 1896, more than 26 per cent of the total catch was found between the depths of 15 and 18 meters—these observations having been made in water 18 meters in depth—and nearly 21 per cent during the first half of June. In 1895, about 17 per cent were found in the bottom water in the first part of June, and nearly 16 per cent during the second half of that month. At the time these observations were made on the history of the crustacea, it was supposed that this swarm of *Cyclops* died, but the observations made in 1905, 1906 and 1907 show that the story is quite different. It appears that many, if not all, of these individuals become encysted and remain in this condition until autumn.

On May 22, 1906, the mud was very carefully examined for cocoons, but none were found, although many *Cyclops* were caught just above the mud. On June 1, cocoons were found in fair numbers and were present in great numbers at all later dates. The cocoon is a small oval body, about 0.65 millimeters in length and 0.50 millimeters in cross diameter. It is composed of particles of the mud, vegetable *débris*, etc., which form the bottom of the lake in deep water, rather firmly cemented together by some adhesive substance. The entire body of the *Cyclops* is concealed by this covering with the exception of the abdominal setae, which are readily seen protruding. The abdomen of the *Cyclops* is usually, though not always, flexed, so that the setae protrude on the ventral side and toward the anterior end. The origin and nature of the cement which holds the particles of mud together have not been determined, but it should be noted that the particles are firmly agglutinated and not merely collected about the animal. The cocoon forms a firm coating, which can, however, be dissected off from the *Cyclops* without difficulty. It is not uncommon to find two cocoons firmly adhering to each other, and clusters of three or four are by no means rare.

The number of these cocoons is very great, although no exact estimate can be given, because it is practically impossible to ascertain the area from which any given quantity of mud has been taken. They seem to lie on the surface of the mud, and the largest number is secured if the hose through which the mud is pumped barely reaches its surface. If the hose is permitted to enter the mud, few or no cocoons may be found in the catch. Some idea of the number may be obtained from the fact that it was very common to find from four to eight or ten in the amount of mud which would be picked up in a medicine dropper and spread out for examination in a small watch glass. Observations were made from time to time during the summer, which showed that the cocoons were present and in the same condition as earlier in the season. Since the absence of oxygen excludes the crustacea from the lower water of Lake Mendota during July, August and September, and since numerous examinations of the lower water were made without finding young *Cyclops* in transit from the bottom to the habitable water, it seems clear that the cocoons are not hatched during this period. It is certain that a considerable number die, since the cocoons may be found with the dead *Cyclops* enclosed.

Careful watch was kept at the time when hatching might be expected—late September and early October. Cocoons were found more sparingly in September, at which time also a few young *Cyclops* were taken in the lower water. This was before the oxygen reached the bottom and indicates that the *Cyclops* were hatching and passing into the habitable water. Cocoons were found as late as October 15, and a single cocoon was found on October 23. A great amount of material was examined on that date but no more specimens were discovered. In middle and late October the lower water is fully occupied by crustacea, and it is not possible to identify those individuals which may have been hatched from cocoons. The facts, however, show that the resting period is about four months in length.

Since the lower water of Lake Mendota loses its oxygen in summer, it was thought that encystation might be induced directly by the decline in that gas. Observation showed, how-

ever, that a large amount of oxygen was present when the animals entered the resting stage. The following table shows the facts:

1906.	Depth, m.	Temp., C.	O, cc. per l.	Remarks.
May 22.....	22	9.6°	5.2	No cocoons.
May 29.....	22	9.6°	4.7	No cocoons.
June 2.....	22	9.9°	4.0	Abundant cocoons.
June 8.....	22	10.1°	3.0	Abundant cocoons.

It appears that there was as much as four cubic centimeters of oxygen per liter when the cocoons were formed. This is a smaller amount than the surface water contained, as this held from six to seven cubic centimeters per liter. This Survey has found, however, that the genus *Cyclops* is by no means sensitive to a decline in oxygen under the ordinary conditions found in lakes, and that it can live in large numbers in water which contains 0.5 cubic centimeters or more of oxygen. These observations do not apply directly to *C. bicuspidatus*, but it is hardly probable that this species differs widely in this respect from its nearest allies. It does not seem probable that the rise of temperature in the lower water determines directly the time of pupation. It will be seen from the table that during the period in which the change took place the temperature rose from 9.6° to 10.1°—a change of only 0.5°.

The hatching in the autumn begins and seems to be almost completed before the oxygen reaches the bottom water in appreciable quantities and while the temperature of the bottom water is not changing greatly and is decidedly higher than it was in June. This will appear from the following table:

1906.	Depth, m.	Cc. O per l.	Depth at which O disappeared.	Temp. at bottom.
Sept. 26.....	22	0.0	15 m.	12.2°
Sept. 29.....	22	0.0	14.5 m.	12.7°
Oct. 6.....	22	0.0	17 m.	12.8°
Oct. 8.....	22	0.0	14 m.	13.0°
Oct. 11.....	22	5.5		13.8°

The cocoons began to hatch before the end of September and almost all had disappeared before October 8.

So far as these observations go, therefore, encystation and hatching are not coincident with any marked physical change in the environment, and must be considered as the result of a general process of development. This conclusion is confirmed by additional observations made in the spring of 1907. The season was unusually cold, the temperature of April in Madison being 4.5° C., and that of May 4.8° below the average of the last twenty-nine years. The lakes warmed slowly, and the quantity of oxygen in the lower water was unusually large. Cocoons were found on May 29, the bottom water containing seven cubic centimeters of oxygen per liter, with a temperature of 9.6°. It will be noticed that the cocoons were found earlier than in 1906, in spite of cooler weather and a greater supply of oxygen.

It should be noted here that Birge is wrong in the statement ('97, p. 326) that *Cyclops brevispinosus* is at all times the dominant species of *Cyclops* in Lake Mendota. The statement was based on the determination of adult females taken from the collections and identified by Dr. Marsh. While *C. brevispinosus* doubtless furnished the majority of females identified, the great mass of immature crustacea in the spring must belong to *C. bicuspidatus*.

A considerable number of cocoons was hatched in the laboratory. Those collected June 4 hatched after a week or more; others collected June 14, hatched on June 19. Those collected in September hatched, on the whole, more promptly, beginning to come out within a day. However, there are always great individual differences in time of hatching, and there have not been enough observations made to determine an average period. In 1907 hatching began within a day or two. The exit of several *Cyclops* from cocoons was observed under the microscope. The cocoon was broken at the rear and loosened by violently extending and flexing the abdomen, and the animal backed out of the part which covers the body. It was positively determined that no moulting accompanies the release from the cocoon nor does any development of the animal take place in the exit. A num-

ber of individuals thus hatched were raised to maturity and sent to Dr. C. Dwight Marsh for identification. He identified them as *C. bicuspidatus* Claus. This is the same species which Dr. Marsh formerly called *C. pulchellus* Koch and so reported in his paper on the plankton of Lake Winnebago and Green Lake, and in other publications. He now agrees with Schmeil in rejecting Koch's name for the species.

Marsh ('03, p. 27) reports that *C. pulchellus* (*bicuspidatus*) is found as a summer limnetic form in the Chain of Lakes, Lakes Geneva, Elkhart, Cedar, Birch, Stone, Sand, Michigamme and Long (Fond du Lac County). It is the common limnetic form in the Great Lakes, and is found in Lake Winnebago from October to June only, being absent in summer. From this statement, and from the facts as ascertained in Lake Mendota, it would appear that the species prefers cool water, that it might live in an active state throughout the year in any lake whose deeper and cooler water was such as to afford it a refuge during the summer, and that it would become encysted in lakes where the oxygen disappeared from the lower water in the summer. The facts, however, are much more complex and puzzling.

This Survey has determined the amount of oxygen in the Chain of Lakes, Lakes Geneva, Elkhart, Stone and Sand. It is abundant throughout the summer, except in Rainbow Lake (one of the Chain of Lakes), where it almost disappears in September, and even in this lake there remains a cool stratum of water with sufficient oxygen. Sand Lake is shallow, only 9.5 meters deep, with a bottom temperature of 18.7° C. on August 22, 1907; only slightly below that of the surface. It is, therefore, of a type wholly different from the other lakes and more closely resembles Lake Winnebago, which is a very large, shallow lake and is nearly homothermous. In one of these lakes, however, the species disappears during the summer, while in the other (Sand Lake) it remains active and no cocoons could be found in mud from the bottom. No cocoons were found in mud from Stone Lake, while mud taken in Rainbow Lake on June 29, 1907, contained them.

Cocoons were also found in several other lakes, as listed in the table below:

	Date.	Depth, m.	Temp., C.	O, cc. per l.
Rainbow	June 29, 1907	27.0	5.6°	0.12
Kawaguesaga.....	Sept. 6, 1906	17.0	11.3°	0.0
Kawaguesaga.....	Aug. 5, 1907	17.0	8.1°	0.0
Black Oak	Aug. 14, 1907	24.5	5.2°	0.6
Thousand Island	Aug. 13, 1907	21.5	4.7°	0.5
Long (New Auburn)....	Aug. 29, 1907	23.0	8.7°	0.2

Kawaguesaga Lake is of the same type as Lake Mendota, the bottom water losing its oxygen in summer. Black Oak, Thousand Island and Long lakes have abundant oxygen in the cold water, and there can be no such direct cause for encystation as appears to exist in Mendota.

The case of Rainbow Lake is very interesting. It is one of the Chain of Lakes, Waupaca, having an area of about 100 hectares (243 acres) and a maximum depth of about 29 meters (95.1 feet). The following table shows the condition of the lake as regards temperature and oxygen. In September the lower water is practically uninhabitable, though there is still a thin stratum of cool water above 10 meters, which contains abundant oxygen. In June, when the cocoons were found, there was abundance of oxygen in the cool water.

Depth, m.	June 29, 1907.		September 10, 1907.	
	Temp.	O, cc. per l.	Temp.	O, cc. per l.
0.....	22.6°	5.8	18.8°	6.1
10.....	10.0°	5.7	10.5°	0.15
15.....	6.5°	4.5	7.0°	0.25
20.....	5.9°	2.2	6.3°	0.02
25.....	5.7°	0.6	6.0°	0.0
27.....	5.6°	0.12	5.9°	0.0

It is plain that there is here no obvious direct cause for encystation. Still further, Marsh ('03, p. 27) places Rainbow Lake in his list of "*pulchellus* (i. e. *bicuspidatus*) lakes," in which

this species is a limnetic form during the summer. It is clear, then, that if these cocoons belong to this species, as they appear to do, part only of the members of the spring broods can become encysted, and other individuals must go on reproducing during the summer. Whether the fall broods come from the encysted individuals or from the others is unknown. It is hard to see why encystation should take place at all in this lake.

It is to be said that, if our species is the same as the European form, its habitat is different. Schmeil ('92, p. 79) speaks of it as coming from "Teichen" and "Wassertümpeln," and other authors give a similar habitat. Marsh, on the contrary, speaks of it as characteristic for the Great Lakes and found in comparatively few inland waters.

Our knowledge of the species, its distribution and its habits, is so scanty and fragmentary that no interpretation of the facts here set forth is possible in relation to the life history of the species. It may be that the form is migrating from the Great Lakes to the smaller waters and is acquiring the power of encystation in adaptation to the ordinary summer conditions of such lakes; or the species may be moving toward the larger and colder bodies of water and losing, in part, in such lakes as Rainbow, the adaptations which were necessary in shallower bodies of water with more abundant plankton.

It appears, therefore, that the encystation of *Cyclops* occurs in lakes of very different types, both as regards temperature and the oxygen content of the lower water. It is at present impossible to correlate the phenomenon with any definite physical change in the lake, or with any known condition of the lower water in summer. If the cocoons all belong to one species, as they appear to, part of the individuals in certain lakes must become encysted and part remain active. It is certain that the phenomenon is not local, as cocoons have been found from six lakes situated in the southern, northeastern and northwestern parts of Wisconsin. The most distant lakes lie at angles of a triangle whose sides are about 225, 190 and 115 miles long.

A number of other interesting questions remains unsettled. Among these are the nature and origin of the glutinous material

which constitutes the cement of the cocoon. Certain crustacea regularly secrete such a substance; among them, *Holopedium*, one of the Cladocera. So far as we are aware, no such substance has been found secreted by a Copepod. The tissue changes during the four months of encystation constitute another subject on which nothing has been done; nor is it known whether or not the encystment is accompanied by moulting.

So far as the authors know, this is the first case of this kind reported from the Copepoda. It opens a new and interesting field of research into the adaptations of crustacea to the different biological conditions offered by inland lakes.

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SOME AQUATIC INVERTEBRATES THAT LIVE UNDER ANAEROBIC CONDITIONS.

CHANCEY JUDAY.

[Biological Notes from the Laboratory of the Wisconsin Geological and Natural History Survey. II.]

During the past two years, many observations have been made on various Wisconsin lakes for the purpose of determining the amounts and kinds of gases dissolved in their waters. Particular attention has been given to the dissolved oxygen, since it plays such an important rôle in the metabolic processes of living organisms, in order to see how these organisms affect its amount and distribution, and, in turn, how its amount and distribution affect the life of the lakes. Birge¹ has given a general summary of some of the results that have been obtained. These studies have been carried on by the Wisconsin Geological and Natural History Survey, and a detailed report of the results is now being prepared for publication.

It is the purpose to deal here with only one phase of the second problem indicated above, that is, how the animal life is affected by the absence of dissolved oxygen. Several lakes have been found in which the lower water is devoid of free oxygen for a considerable period during the summer. In some lakes this condition may exist from the middle or last of May until the early part of October, while in others it may not last more than one or two months. This lower stratum of water is cooler than the upper, hence its density is greater. Because

¹ Birge, *Trans. Amer. Fish. Soc.*, 1906, pp. 141-161.

of this difference in density, the upper water is not mixed with the lower by the wind but floats upon the latter as a separate and distinct body of water, cutting off the lower water from contact with the air. Thus, there is no opportunity for the lower water to renew its supply of dissolved oxygen when this is once exhausted. That is, its supply is limited to the amount of oxygen obtained during the spring overturn, and when this supply is exhausted, it remains free from dissolved oxygen until the fall overturn. The free oxygen is used up in part by the animals which inhabit this region, but by far the greater part of it is consumed in the decomposition of the various plants and animals, chiefly plankton forms, which die, sink down into this region and decay.

When it was found that anaerobic conditions existed in this lower water, the question of the effect of this on the animal life of the region at once presented itself. So far this question has been studied chiefly on Lake Mendota, where the lower water is devoid of dissolved oxygen for about three months during the summer. In order to be sure that this water contained no free oxygen, it was tested by three different methods, two titration methods and by boiling the water and analyzing the gas obtained. All gave negative results.

Up to the beginning of this anaerobic condition, the lower water is usually inhabited by large numbers of plankton crustacea; but as soon as this condition is once established, crustacea are no longer found in this region, except perhaps an occasional individual which is either dead or shows evidences of disease or senility that would soon prove fatal. Larval *Corethra*, however, was found as a regular inhabitant of this water which contained no dissolved oxygen. Professor Needham thinks it is the larva of *Corethra punctipennis*, but no adults have yet been reared, so that the species has not been definitely determined. It seems to experience no difficulty in occupying this region, even when the water has a very offensive odor. In fact, one was invariably safe in predicting very little or no oxygen at depths from which this form was obtained in the daytime. There is a difference of opinion among authors as to whether the two pairs of air sacs possessed by this larva

have a respiratory function or not. In this case, however, one can readily see how they may fulfill a very important respiratory function by serving as reservoirs for oxygenated gas. This would enable the larva to remain in water that is devoid of free oxygen for a considerable period of time without inconvenience. Since it is a very good swimmer, it can readily reach water that possesses some free oxygen whenever a new supply is needed. It is not yet clear just why the larva should choose to remain down in this region during the day. It seems to react negatively to light, as it moves down into the lower water very early in the morning and remains there during the day. At night, however, it moves up into the upper water and is very often found at the surface.^{2 3} One would scarcely expect light alone, however, to cause the animal to descend to a depth of 12 or 15 meters, since the transparency of the water is tolerably low, a Secchi's disk generally disappearing from view at a depth of two or three meters. But larval *Corethra* is the only form which inhabits this region, so it is entirely free from enemies there. It seems probable that this may be one of the reasons why it remains there during the day, when it would suffer most from animals which prey upon it. At night, when it would be comparatively free from the attacks of enemies, it rises into the upper water where it may obtain an abundance of oxygen and food, both of which are absent from the lower water.

Several forms were also found in the muddy ooze at the bottom of the lake, although no free oxygen was present there. The mud was obtained by means of a pump and hose and was pumped through bottles that could be sealed and kept air-tight while they were being conveyed to the laboratory for study. Thus the material did not come into contact with the air until removed from the bottles for examination. Protozoa were found to be the most common forms. Living, active representatives of the following genera were noted:—*Pelomyxa*, *Difflugia*, *Colpidium*, *Gyrocorys*, *Peranema*, *Coleps*, *Paramecium*,

² Birge, *Trans. Wis. Acad. Sci., Arts and Letters*, vol. 11, p. 410. 1897.

³ Juday, *Ibid.*, vol. 14, p. 556. 1904.

Prorodon, *Lacrymaria*, *Uronema* and *Monas*. All of these forms were perfectly normal. They did not show any unusual vacuolation, nor any other visible evidences whatever of ill effects resulting from the anaerobic conditions under which they are living. This result does not agree with some of those that have been obtained in experiments along this line. Budgett⁴ found that the membrane in many Infusoria bursts and the liquid contents flow out when they are subjected to anaerobic conditions. Pütter⁵ observed characteristic changes in the protoplasm of *Paramecium* and *Spirostomum* when they were placed in an atmosphere of nitrogen. Loeb and Hardesty⁶ also observed that amoebae and *Paramecium* became vacuolated and finally burst when deprived of free oxygen. In the experiments, however, the change to anaerobic conditions was made rather suddenly, so that the Protozoa did not have time to adjust themselves gradually to the new conditions. This sudden change to an atmosphere of nitrogen or hydrogen, in which the experiments were performed, affected the equilibrium of the gases contained in the Protozoa and doubtless resulted in a rather rapid diffusion of gases into and out of them which may have been largely responsible for the deleterious effects that were observed. In the lower water of Lake Mendota, however, the change to anaerobic conditions is gradual, thus giving the Protozoa time to adjust themselves to the change, and they show no visible effect resulting from it. Neither was there any noticeable effect when they were changed back again to aerobic conditions by exposing them to the air.

All of the above Protozoa seemed to experience no difficulty in withstanding this anaerobic condition for a considerable period of time. They were still found as active and in as great abundance in late September and early October, after the water had been free from dissolved oxygen for nearly three months, as in early July at the time of its disappearance. Pütter⁷ also found that he could keep specimens of *Nyctotherus*

⁴ Budgett, *Amer. Jour. Physiol.*, vol. 1, p. 210. 1898.

⁵ Pütter, *Zeitschr. f. allg. Physiol.*, Bd. 5, pp. 592, 596. 1905.

⁶ Loeb, "Dynamics of Living Matter," p. 21. 1906.

⁷ Pütter, *Zeitschr. f. allg. Physiol.*, Bd. 5, p. 583. 1905.

cardiformis for fifty days in a medium that contained no free oxygen when he fed them on egg albumen. No chlorophyl was found in any of the Protozoa that were obtained from the bottom of Lake Mendota, so that they could not have obtained free oxygen by splitting up CO_2 . Even if they had contained chlorophyl, the intensity of the light at a depth of 20 or 22 meters was undoubtedly not sufficient to make it serviceable, because the transparency of the water was low. The medium in which these Protozoa lived was not only free from dissolved oxygen, but the water also contained about 7.5 cubic centimeters of free CO_2 per liter and about 5 or 6 cubic centimeters of CH_4 .

Some higher invertebrates were also found in the mud. The worms were represented by specimens of *Tubifex*, *Limnodrilus* and *Anguillula*; the rotifers by *Chaetonotus*; the crustacea by an Ostracod belonging to the genus *Candona*; the insect larvæ by a large red Chironomid larva; and the mollusca by *Corneocyclas idahoensis*.

In order to study the activities of these forms while they were in a medium which contained no dissolved oxygen, some of the muddy water containing them was pumped into glass jars that could be sealed and kept air-tight. Great care was exercised in taking the samples so as to keep the medium free from dissolved oxygen. The muddy water was pumped through the jars so that they could be thoroughly flushed and all of the material removed that had been exposed to the air. After being sealed, the jars were taken to the laboratory and placed in a refrigerator in order to keep the temperature about the same as that of the mud in the bottom of the lake. They were kept at a temperature of 13° to 15° C., which was 1.5° to 2.5° higher than the temperature of the mud in the lake at the various times the samples were taken. The jars were removed from the refrigerator from time to time and carefully examined to see how active the various animals were. The worms were as active under anaerobic conditions as they were when placed in aerated water. So far as could be determined, they were not affected in the least by the lack of free oxygen. This result should probably have been expected, as so many of

the parasitic worms have adapted themselves to a life under anaerobic conditions. It has been found that some of the parasitic forms living in the absence of free oxygen have a very high glycogen content, and it would be interesting to know whether this is true of these non-parasitic forms, and also whether there is any change in the relative amount of glycogen during the aerobic and anaerobic periods.

Chaetonotus was always active. *Candona* kept itself buried in the mud and seemed to show very little activity, but when placed in aerated water it soon became much more active. Chironomid larvae were scarce, and no specimen of them was found in the jars on which observations were made. *Corneocyclas* remained quiescent with the valves tightly closed. Some of the jars were kept under observation for two weeks and no living specimens were ever found with the valves open. The mud was also carefully examined for indications of activity, but no evidences of it were found. But when removed from the jars and placed in aerated water, or when the contents of the jars were poured into crystallizing dishes and exposed to the air, they soon became very active. These results seem to indicate that this mollusk remains quiescent or dormant during the period when the muddy ooze at the bottom of the lake contains no dissolved oxygen. This means, then, that this "latent life" condition continues for a period of about three months each summer in Lake Mendota. In 1906, the earliest date on which the bottom water was found to be free from dissolved oxygen was July 9, and it remained in this condition until October 9. This covered most of the time which one would naturally consider most favorable for growth and activity, because of the higher temperature of the water, but *Corneocyclas* seems to have adapted itself to a quiescent period at this time because of the lack of dissolved oxygen. The time at which it reproduces has not been determined. Neither has there been any opportunity as yet to ascertain how widely it is distributed over the bottom of the lake. It may extend into the shallower water, where it would find a sufficient amount of free oxygen to enable individuals in this region to continue their activities during the summer. If reproduction takes

place during the summer, the oxygen conditions in the shallower water would not interfere, and those individuals that are found in the deep water may be the descendants of those nearer the margins of the lake. Then, too, the reproductive period may come in deep water in the early part of the season before *Corneocyclas* enters its quiescent period, and both temperature and oxygen conditions are very favorable just after the fall overturn for a reproductive period at that time.

DISSOLVED OXYGEN AS A FACTOR IN THE DISTRIBUTION OF FISHES.

CHANCEY JUDAY AND GEORGE WAGNER.

[Biological Notes from the Laboratory of the Wisconsin Geological and Natural History Survey. III.]

Lakes of various sizes and depths are numerous in the central portion of northern Wisconsin, and the good fishing for which some of them are noted attracts many anglers each season. For the purpose of improving and diversifying the fishing, efforts have been made from time to time during the past few years to stock most of the lakes that are of any considerable size and depth with the Great Lake or Mackinaw trout (*Cristivomer namaycush*). It has been found, however, that this trout does not thrive in many of the lakes in which it has been introduced; in fact, the efforts have met with success in only a very small number of cases. So many failures, of course, raised a query as to their cause. For the past two years, this Survey has been making a study of some lakes in central and southeastern Wisconsin with respect to the gases dissolved in their waters, and the results obtained for dissolved oxygen in some cases suggested that this gas might be a very important factor in the success or failure of the experiments with the Mackinaw trout in the northern lakes. Accordingly, early in September 1906, two of them, Kawaguesaga and Trout lakes, both located in the vicinity of Minocqua, were visited, and observations were made on them relative to the amount and distribution of the oxygen dissolved in their waters.

Trout Lake consists of two parts connected by a comparatively narrow but deep channel. The south part is about 4 kilometers (2.5 miles) long and 2.4 kilometers (1.5 miles) wide, while the north part is only about half as large. Both parts have a maximum depth of about 30 meters (100 feet). Samples of water taken at a station in the deepest portion of the south part of the lake showed a considerable amount of oxygen in the lower water. Half a meter above the bottom, where the lake was 29.5 meters deep, the water contained 0.9 cubic centimeters of dissolved oxygen per liter, and at 27 meters there were 2.1 cubic centimeters, which was a little more than a third of the amount found at the surface (5.6 cubic centimeters).

Kawaguesaga Lake is very irregular in outline and consists of three basins which are connected by rather narrow channels. The eastern part is the largest and deepest, having an area of about 2.6 square kilometers (1 square mile) and a maximum depth of about 18 meters. An examination of the water of the eastern portion showed that it contained only a trace of dissolved oxygen at 10 meters and none at all at and below 10.5 meters. That is, all of the cold, lower water was devoid of free oxygen. Gill nets were set in various parts of Kawaguesaga Lake and at various depths also, but no specimens of Mackinaw trout were obtained. So far as could be learned, also, it has never been caught in this lake by fishermen. This seems to indicate that conditions here are unfavorable for it. On the other hand, six Mackinaw trout were caught in 24 hours when the gill nets were set within about a meter of the bottom in the deepest portion of the south part of Trout lake. No specimens of it were caught, however, when the nets were set in the warm, upper water, but only when they were placed in the cool, lower water of the deeper portions. The best results, in fact, were obtained by placing the nets near the bottom where the water was nearly 30 meters deep.

The fishermen stated that the Mackinaw remains in the deep water of Trout Lake during the entire summer, and these results confirm their observations for late summer at least, and, so far as could be ascertained, there is no evident reason why this

should not hold true for the entire summer. This habit, then, of remaining in the cool, lower water during the summer means that this region of a lake must be habitable for the trout at this period or it cannot be successfully acclimated. The chief factor in making this region habitable is dissolved oxygen. If the free oxygen entirely disappears from the cool water in summer, or if it is reduced to an amount so small that it will not supply the needs of the fish, it cannot occupy this region, and therefore will not thrive in such a lake. This readily explains why the efforts to stock Kawaguesaga Lake with Mackinaw trout proved unsuccessful. As stated above, it was found that all the cool, lower water of this lake was devoid of free oxygen in early September, and this condition doubtless exists for a considerable period each summer. The trout is unable, therefore, to occupy the cool water, and this makes conditions so unfavorable that it cannot maintain itself in the lake. On the other hand, the lower water of Trout Lake contained enough dissolved oxygen to supply the trout's needs, and trout are found here in abundance. They are native fish, not the result of artificial planting.

In order to study the effects of such water on active fishes, some experiments were performed on perch (*Perca flavescens*) and crappies (*Pomoxis*), as they are very hardy fishes. Some of the lower water in Mendota which was free from dissolved oxygen was pumped up into aquaria and specimens of these fishes were immediately placed in these aquaria. In every case they died in a very short time, the length of time varying somewhat for the different individuals. In general, also, the perch lived a little longer than the crappies. These results only confirmed what had been taken for granted before performing the experiments, that is, that the lower water is uninhabitable for fishes when it is devoid of free oxygen. Their inability to occupy this region means a very considerable restriction in their vertical distribution in some lakes. In some of the smaller lakes which are well protected from winds and which contain a considerable amount of decaying organic matter, the dissolved oxygen may entirely disappear at a depth of 5 or 6 meters in August, so that the fishes would be limited to a warm, upper strata-

tum of water only 5 or 6 meters thick. Even in so large a lake as Mendota, which has an area of about 39 square kilometers (15 square miles), there is practically no oxygen below a depth of 10 or 12 meters in August and early September. As the lake has a maximum depth of only about 25 meters, this means that half the maximum depth is uninhabitable for fishes at this time. Scarcely more than a third of the maximum depth of some lakes can be occupied in late summer. These unfavorable conditions, which confine the fishes to the warm, upper water during the latter part of the summer, may be very largely responsible for some of the epidemics which result in the destruction of large numbers of them.

So far as the authors know, no fish has ever been found which leads either an active or a passive life in water that is free from dissolved oxygen. One fish is known, however, which passes through a "latent life" period. *Protopterus annectens*, which inhabits shallow, muddy water and swamps in tropical Africa, burrows into the mud, encloses itself in a sort of cocoon and passes the dry season in a dormant condition. But this cocoon contains a respiratory funnel which enables the fish to obtain air for respiration during this period. With such a remarkable adaptation as this represented in the class of fishes, it seems entirely within the range of possibilities that some member could adapt itself to a period of passive life, at least in water that contained no free oxygen.

In this connection, it is also interesting to note that the removal of the dissolved oxygen from the lower water of a lake is due chiefly to the decay of organic matter. This decaying material is supplied for the most part by the microscopic organisms living in the water. At their death, these organisms sink to the bottom, decaying on the way down as well as at the bottom, thus removing the free oxygen from the lower water. Obviously, the rapidity with which this oxygen disappears depends mainly upon the amount of decaying material which sinks into this region, which, in turn, depends chiefly upon the quantity of plankton growing in the lake. Because of their short life and the rapidity with which they reproduce, the plankton algae are much more important factors in supplying

the decaying material than the zooplankton forms. The plankton organisms, either directly or indirectly, constitute a very important element of the food of most fishes at some stage of their development; in fact, it has been asserted that the production of fish is correlated with the amount of plankton produced by a stream, or lake. But the results obtained on a number of Wisconsin lakes show that, beyond a certain limit, the increase in the amount of plankton would tend toward a decrease in the production of fish in these lakes, especially of some species, rather than an increase, by making conditions unfavorable for them. This upper limit would vary somewhat for different lakes. A very large growth of plankton in a lake would furnish an abundance of decaying organic matter which would soon use up the dissolved oxygen in much or all of the cool, lower water, and this would mean a very considerable restriction in the horizontal distribution of the fishes. Under these conditions, they could live only in the warm, upper water, and a species that required cool water would not be able to live in such a body of water.

So far as oxygen conditions in the lower water are concerned, a lake that is poor in plankton would be best adapted to fish life in that region, but the question of food now enters as a factor of equally great importance. As indicated above, plankton plays such an important rôle as a fish food that a scanty supply of it would mean a scarcity of proper food. This condition would be accompanied by the usual results, few fish and these in poor physical condition. Thus the results obtained in these studies seem to indicate that the lake that is best adapted to fish life is one that contains neither an unusually large amount of plankton, which would cause the rapid and early depletion of the dissolved oxygen in the lower water in the summer, nor an amount so scanty as to endanger the food supply, but a medium amount. However, each lake possesses features peculiar to itself, and there are factors involved which make it impossible to give any general rule as to quantity that would apply to all lakes. These individual differences must be determined for each lake.

In conclusion, then, it may be said that the amount of dissolved oxygen in the lower water of any lake determines whether fishes that require cool water during the summer can or cannot thrive in a lake, and it also determines the vertical distribution in a lake of fishes that are able to occupy either warm or cool water.

NOTES ON THE FISH FAUNA OF LAKE PEPIN.

GEORGE WAGNER.

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Lake Pepin is an expanded portion of the Mississippi River, on the southern boundary of the counties of Pierce and Pepin, Wisconsin. The cause of this expansion is the deposition of material by the Chippewa River at the point where it joins the Mississippi. The Mississippi here was not able to carry this extra load. As a consequence, it has been deposited as a sort of wing dam, increasing with time and finally forming a considerable barrier against the waters of the Mississippi. These, so checked, collected above to form a lake, until a point of equilibrium was reached. At present, the height of the lake varies through a range, as far as the extremes on record are concerned, of about nineteen feet, and through a range of four or five feet during an average season.

Lake Pepin is sigmoid in outline, extending from northwest to southeast. Its length along the center line is about twenty-five miles; its width varies from one-half mile to two and one-half miles. In the main, its shores are steep bluffs; these recede from the Minnesota side for several miles at Lake City, and on the Wisconsin bank near the lower end of the lake at Pepin.

At the upper end of the lake, for a mile or more, the bottom is in large part mud. The rest of the lake bottom is chiefly sand, with small patches of sand and mud occurring at rather regular intervals. The depth at the upper end ranges

from ten to twenty feet as maxima along the lines of sounding, but gradually deepens as one goes toward the lower end. At the boundary between Pierce and Pepin counties the maximum depth is thirty-two feet. At Lake City, Minnesota, it is thirty-seven feet very close to the government light. At the lower end of the lake, the maximum depth of fifty-six feet is found close to the Minnesota shore, while the Wisconsin half of the lake, and the river below, are very shallow, probably due to the checking action of the Chippewa River, which enters the Mississippi from the Wisconsin side, about one mile below the foot of the lake.

The chief source of the water of Lake Pepin is of course the Mississippi itself, but it also receives considerable volumes from the Isabel and Rush rivers and Pine Creek, on the Wisconsin shore, and from a creek just north of Lake City on the Minnesota side.

Let it be stated right here that Lake Pepin is a lake in every sense. The very accurate surveys of the Mississippi River Commission have disclosed no difference of level between the two ends, nor any perceptible current. The lake itself has a considerable retarding effect on the Mississippi above it; the river near the lake has a fall of a little less than one-tenth of a foot to the mile.

We have here, then, a typical lake, one of the largest in Wisconsin (excepting of course the Great Lakes) and moreover one whose general physical characters are not only very well known, but are constantly being kept track of. This is entirely due to the fact that the lake is part of the channel of the Mississippi River, and as such a highway for steamboat transportation. The Mississippi River Commission has constructed a map of the lake in three sheets, on a scale of one to twenty thousand, or about three and one-quarter inches to the mile. These maps give contours as well as other natural and cultural features of the shore. At intervals of rather less than a quarter of a mile, there are lines of soundings from shore to shore, soundings along each line being indicated at intervals of about one hundred and fifty feet. The character of the bottom is also frequently noted. The Commission maintains

gauges at Lake City and at many points on the Mississippi above and below.

This lake, it seems to me, offers a very favorable place for the prosecution of a systematic biological survey. I know of no other lake in the Mississippi valley of which the physical features are so well known. There remains, however, even here, much work to be done on temperatures and other features.

Furthermore a thorough study of this lake is highly desirable, for, as I hope my notes on the fishes will show, it is a great field for commercial fisheries, and by proper procedure its importance could be further increased. It seems to me that it would be well worth while to experiment with the introduction here of certain food fishes now not existing here, especially of certain whitefish; so far as I am able to ascertain, no such experiments have ever been made. In Lake St. Croix, somewhat further north, at the mouth of the St. Croix River, the United States Fish Commission planted two million whitefish fry in 1891-2 (*Rept. U. S. F. C.*, vol. 18, p. lxxxiii). In 1892-3 it planted in the same lake two thousand three hundred yearlings of lake trout (*Rept. U. S. F. C.*, vol. 19, p. 134). Lake St. Croix is a smaller and decidedly shallower body of water than Lake Pepin. Yet an occasional report of the capture of a lake trout in this region of the Mississippi seems to indicate that the experiment was not entirely a failure. A repetition of it in Lake Pepin, with due care in details and followed persistently for several years, would very probably prove a success.

It is as a first contribution toward a biological survey of Lake Pepin that I offer the following notes on its fish fauna.

In the summer of 1903, the writer spent two weeks at Lake City, Minnesota, engaged in collecting certain anatomical material. In 1904, he spent nearly three months at the same place, as an employe of the United States Bureau of Fisheries, studying the biology of *Polyodon*. During both periods, collections and notes of the fishes occurring here were made, as time allowed. During both periods he made much use of the aid, freely given, of Mr. Christ. Schnell, of Read's Landing, Minnesota, a man who has fished in these waters for many

years and who has a remarkably wide and accurate knowledge of the fishes of the region. The writer is greatly indebted to him for many favors. To the United States Bureau of Fisheries, and especially to Dr. W. B. Evermann, he also owes much, and Dr. Meek of the Field Museum kindly named some of the forms and corroborated the determinations of a few others.

The material comes mainly from three sources: First, an eighteen hundred foot seine of six inch mesh, and about twenty feet in depth, hauled twice a day, during my stay, by Mr. Schnell and his crew. Secondly, a Baird seine, seventy-five feet in length, sometimes hauled separately, but more frequently attached behind the large seine when this had been spread eight or nine hundred feet from shore. In this manner we obtained several forms which would otherwise have been missed. Lastly, a thirty foot minnow seine was hauled a number of times daily along shore at various places in the neighborhood of Rest Island, the high shore just north of Central Point (see Chart No. 184, Mississippi River Commission). The number of hauls made by all these seines gives some reason for believing that we saw practically all the species occurring with any frequency in the lake. A few more species would probably be found by examining the muddy upper end of the lake.

In the enumeration below, I have followed, without deviation, the nomenclature of Jordan and Evermann's "Manual." The common names, when given, are those used by the fishermen of the region.

1. *Ichthyomyzon concolor*. Lamper Eel. This is an extremely common form here. It attacks the catfishes, and in much lesser degree the buffaloes and suckers, but its favorite host is *Polyodon*. Among about fifteen hundred spoonbills examined, there were but few that did not show some signs of lamprey bites. A number of times I have taken from ten to twenty-five lampreys from one specimen, although they usually desert their host when brought into very shallow water. They attack all parts of the host, most frequently however the belly. Occasionally one was found to have worked its way under the gular membrane and to have attached itself to the

isthmus, its body being entirely invisible until the pouch was examined.

2. *Ichthyomyzon castaneus*. Lamper Eel. Unfortunately, I did not preserve the necessary specimens to prove the presence of this form. From specimens examined on the spot, I feel confident that forms which would be referred to this species occur. From some observations made, I incline, however, toward the belief that *I. castaneus* is not specifically distinct from *I. concolor*. I hope soon to be able to collect the necessary material for deciding this point.

3. *Polyodon spathula*. Spoonbill. One of the most abundant forms in Lake Pepin throughout the summer, and probably throughout the year. It is caught in large numbers in the seines. The writer has seen as much as fifteen hundred pounds brought in at a single haul of the big seine. Seemingly the spoonbill is of a rather roving disposition, cruising up and down the lake in large schools. Hence its appearance in the seines is rather irregular. There may be an abundance one day and practically none the next. In the two years I observed it, it disappeared, or at least its numbers greatly decreased, for a period of about a week in August. This disappearance can have nothing to do with its breeding habits, for the females are not at this time heavy with eggs. It may be simply a seeking of deeper and cooler waters at the foot of the lake.

After much hard work, I have been unable to get any light whatever on the breeding habits of this peculiar form. Dr. Evermann (*Bulletin U. S. F. C.*, vol. 27, p. 284) reports nearly ripe females as occurring in the Ohio River at Louisville in the middle of May. From this one would expect spawning to occur in our region not before the end of June, and more probably in July or August. In the summer of 1904, I examined anatomically every specimen of spoonbill brought ashore at Lake City, about fifteen hundred in all. A fair proportion, whose number unfortunately was not accurately recorded, were recognizably females. But between June 11 and September 1 I did not discover a single female with ripe or nearly ripe spawn, or one that showed signs of

having recently spawned. The fishermen profess to know that spawning takes place in February and March, and the condition of the ovaries in summer lends some weight to this contention. But a female with ripe eggs from this region is yet to be found, although several fishermen have promised me to be on the lookout for them.

Contrary to the usual belief, the spoonbill, in Lake Pepin at least, is in no sense a mud-loving fish. It is never found in the muddy bayous, or close in to shore. To capture it, the seine is carried out from seven hundred to a thousand feet, mid-channel, where the water is fairly clear and the bottom sandy. This makes very improbable also the supposed use of the snout or rostrum for stirring up the bottom. The food of *Polyodon* adds weight to this contention. It consists, in Lake Pepin, entirely of plankton material, in largest part of entomostraca, but not unmixed with algae. There is one seeming, but only seeming, exception to this. Occasionally one finds the specimens of a morning's catch largely gorged with larvae of Ephemerids. But in every such case it was found that Ephemerid imagines appeared in vast numbers the same evening. It appears plain, therefore, that the larvae taken by *Polyodon* were captured on their journey to the surface of the water. During this time they form an integral part of the plankton. Finally, a study of the rostrum itself discloses that its external texture is such that, if used for rooting on the gravelly and sandy bottom of Lake Pepin, it would soon become abraded, but I have never discovered any sign of this in any living spoonbill. Injuries there are on the rostrum frequently enough, but they consist mainly of notches on the side. I suspect that they are caused by the bites of turtles.

On the other hand, the good nerve supply of the rostrum, and the way in which it is fairly paved with sensory end organs, make it patent that it serves a sensory and not a motor function. It probably serves to disclose the denser swarms of plankton, which are then scooped up by the enormously expandible mouth.

Polyodon lives, as noted before, in deep water. But in summer, at least, it remains near the surface. Two things give

evidence of this. In the seining operations it is continually apparent that such spoonbills as become gilled in the meshes of the seine always are found very close to the cork line, showing that they were near the surface when gilled. The second line of evidence concerns their feeding habits. The specimens I examined were brought to shore usually at about eight or nine o'clock in the morning, and about four o'clock in the afternoon. The stomachs of the former were usually gorged with plankton, those of the latter were, with a few exceptions, empty. It would appear, then, that *Polyodon* feeds during the night or very early in the morning. Now, while no accurate determinations of the plankton of Lake Pepin have been made, it is fair to presume that at night the plankton organisms approach the surface more nearly than in daylight. It would be very interesting in this connection to determine the gas content of the deeper water during August, as has been done for other lakes of our state by Professor Birge and Mr. Juday.

Polyodon, more than any other fish in Lake Pepin except *Pomolobus chrysochloris*, has the habit of leaping up out of the water. It leaps with great force, specimens four feet or more in length often leaping clear above the surface, then falling usually on their side. This also occurs commonly far from shore; only once have I observed it close in. This jumping of *Polyodon* is commonly said to be for the purpose of removing the lampreys. This is probably true; at all events, it can easily be observed that the lampreys are knocked off in this manner, sometimes in considerable numbers.

As to its commercial status, the spoonbill is the fish most sought for by the seiners on Lake Pepin. It is only within comparatively recent years, however, that its food value has been recognized. In the last few years it has brought a price nearly equal to that of sturgeon. This, combined with the numbers which are caught, makes it a very profitable fish. There is, therefore, some danger that its numbers may soon be decimated as those of the sturgeon have been. Our present absolute lack of knowledge of its breeding habits makes proper methods of protection a difficult problem. The roe forms no part of the commercial value of the fish here.

Considering the fate of our sturgeons at the hands especially of the French ichthyologists, it is remarkable that we have not a larger synonymy of *Polyodon*. In its dimensions it varies considerably, and in the dimensions of the rostrum this variation is extreme. Specimens are often seen in which the rostrum is very long and slender; in others its width is fully equal to one-third the length, while all intermediate forms occur.

All manuals state definitely that *Polyodon* has no barbels. But it was pointed out by Allis (*Zool. Jahrbücher, Anat.*, vol. 17, p. 671) and discovered independently by myself (*Science*, N. S., vol. 19, p. 554) that these exist. In fifteen hundred spoonbills examined by me, I did not find a single specimen in which they were absent, so they must be considered a constant character. Their size is, however, very variable.

As is well known, specimens of spoonbills below a foot in length are extremely rare. In the fall of 1904, I purchased from Mr. Kane of Bay City, Wisconsin, three specimens all smaller than any I have seen elsewhere, and the smallest probably as small as any now in collections. A few notes on these may not be without interest.

Specimen A. Not in very good condition. Length, 260 mm. to tip of dorsal fork of tail; 215 mm. to base of tail. Distance from tip of rostrum to median point of upper tip, 96 mm. Greatest width of rostrum, 25 mm. The eyes point distinctly toward ventral side. Edge of rostrum very thin and flexible, rayed ossicles only feebly developed. Sensory organs well developed on dorsal side, only feebly on ventral side. Upper jaws dentate only on anterior half, lower jaw dentate throughout. Teeth show distinct tendency toward arrangement in two parallel rows.

Specimen B. Length, 254 mm. to base of tail. Tip of rostrum to median point of upper lip, 98 mm. Greatest width of rostrum, 30 mm. Eyes as in *A*. Sense organs much better developed, especially on gular surface, where they show but little in *A*.

Specimen C. Length, 247 mm. to base of tail. Tip of rostrum to median point of upper lip, 109 mm. Central axis

of rostrum very much heavier than in *B.* Skeleton of dorsal surface of head narrower.

4. *Acipenser rubicundus*. Rock Sturgeon. Pound for pound, this is the most valuable fish marketed by the seiners. Large specimens have become scarce, however, in late years, so that twelve or fifteen in a season are considered a good catch. Smaller ones, up to fifty centimeters in length, are not uncommon. The food of this fish in Lake Pepin, in summer at least, consists entirely of the larvae of Ephemerids.

5. *Scaphirhynchus* (?). White Sturgeon; Sand Sturgeon. From descriptions of the fishermen, it seems certain that there occurs here, though rarely, either *Scaphirhynchus platyrhynchus* Raf., or the recently described *Parascaphirhynchus albus* Forbes and Richardson. I have not myself seen any specimens taken here.

6. *Lepidosteus osseus*. Billy-Gar; Garfish. Seemingly a common form, although only one large female was caught in the big seine. Small specimens, from eleven to twenty-two centimeters in length, were caught almost daily with our minnow seine.

7. *Lepidosteus platostomus*. Billy-Gar; Garfish. Two females, each about sixty-five centimeters in length, and both heavy with eggs, were brought in with the large seine. One dead specimen was picked up on shore. Fishermen do not distinguish between the two species of *Lepidosteus*. *L. platostomus* is seemingly rare, and is looked upon as abnormal.

8. *Amia calva*. Dogfish. Quite common, some being brought in at almost every haul of the seine. Though not considered as of much value as food fishes, they are sometimes shipped with other rough fish, and as no protest ever comes from the commission merchants, they probably find a market.

9. *Ictalurus punctatus*. Blue Cat. A fairly common fish here, though large specimens are not numerous enough to form any considerable proportion of the daily catch. Smaller specimens, up to thirty centimeters in length, were caught in great numbers in our Baird seine. This fish is highly prized and well paid for on the Chicago market.

10. *Leptops olivaris*. Mud Cat. Not so numerous as the

blue cat, nor so highly esteemed; but the two species are not kept separate in shipment to the market.

11. *Schilbeodes gyrimus*. The only bullhead (as all small Siluridae are called in this general region) that we caught was a single specimen of this species, three centimeters in length.

12. *Ictiobus cyprinella*. Buffalo. Very abundant. Caught and shipped in larger quantities than any other fish. When the price warrants, it is dressed by splitting up the back before shipping; otherwise it is shipped "in the round" together with carp, suckers, etc.

13. *Ictiobus urus*. Deep-water Buffalo. During the summer of 1904, I attempted many times to select specimens which I could assign to this species, but without success. Fishermen point out what they consider a distinct "deep-water buffalo," but in no case was I able to distinguish them by any constant characters from *I. cyprinella*. At present I am very much inclined to doubt the distinctness of the two species. However, I hope to be able before long to return to this field and gather more detailed evidence on the subject.

14. *Ictiobus bubalus*. Small-mouthed Buffalo. Occurs very much less abundantly than *I. cyprinella*. As market fish the two forms are not kept separate.

15. *Carpiodes velifer*. Quillback. Not very abundant, but one or two appeared in the big seine almost daily. As elsewhere, this form is very variable, and fishermen apply various names. All specimens of *Carpiodes* examined by me, however, could be referred to this species.

16. *Catostomus commersonii*. A number of young specimens were taken, near the mouth of the creek at the south end of Rest Island. No large specimens were found, and the species probably belongs to the creek rather than to the lake.

17. *Moxostoma anisurum*. Red Horse. A single large specimen and a number of young were taken.

18. *Moxostoma aureolum*. Red Horse. Not at all common. In fact, to one who knows the abundance of Catostomids in the lakes and rivers of Minnesota tributary to the Mississippi, their comparative rarity in Lake Pepin, with the exception of *Ictiobus*, comes somewhat as a surprise.

19. *Cyprinus carpio*. German Carp. Before 1903 the carp was not taken in Lake Pepin in any large quantities. In that year they appeared abundantly, more so in 1904, and they have been on the increase ever since. They were not by any means welcomed by the fishermen. They occur everywhere in such abundance that their appearance on the market has a depressing effect on the price paid for buffalo, for which they form a rather poor substitute. They do present one advantage, in so far as their scales come off much less easily than those of the buffalo, and they are otherwise less sensitive, so that many more can be kept alive in a fish-box, and for a much longer time.

20. *Notropis blennius*. A number of specimens, really too young for satisfactory determination, seem to belong here.

21. *Notropis hudsonius*. Shiner. This is by far the commonest minnow in Lake Pepin, great numbers being taken at every haul of the minnow seine.

22. *Notropis whipplei*. Only a single specimen occurs in our collections.

23. *Notropis rubrifrons*. A common minnow, usually found with *N. hudsonius*.

24. *Hybopsis storerianus*. Numerous specimens were brought in one day in the Baird seine, from mid-channel. The largest was 12 centimeters in length. Teeth, 1, 4-4, 0.

25. *Anguilla chrysypa*. Eel. Eels are said to be common in this region, but naturally our ordinary methods of fishing failed to secure any. I obtained a single specimen by means of a brook fyke set at the bottom in thirty feet of water.

26. *Hiodon tergisus*. Mooneye. Very common, but not often taken in the large seine, because of their small size. On one occasion an immense school was brought very close to shore by the seine, but when in shallow water practically all escaped through the meshes. Locally it is used for food, only the dorsal part being retained in dressing.

27. *Dorosoma cepedianum*. Norwegian Herring. Not very common, and never marketed. The name "Norwegian herring," invariably applied to this form in this region, seems to be a very local one; at least I have never heard it elsewhere.

Its use probably expresses a superficial resemblance between this fish and some form familiar, in the fatherland, to Norwegian immigrants. An old Norwegian fisherman told me his experience with this fish in his early days in America. He recognized it as a herring similar to those he had caught and salted down for winter use in Norway. Hence he proceeded to salt down *Dorosoma*. His description of the odors produced when he tried to cook his salted fish was decidedly more expressive than elegant.

28. *Pomolobus chrysochloris*. Skipjack. Exceedingly abundant, but never marketed, because worthless. Its peculiar habit of leaping out of the water while pursuing its prey is very apparent here, especially at dusk, when the splashing of many specimens is almost continuous until long after dark. I was never able to hook one in Lake Pepin, but while fishing for black bass in the swift waters of the Mississippi several miles below the lake, I was forced to abandon minnows as bait, as the skipjack took all of them. Although worthless as food, the skipjack is not to be despised as a game fish. I consider him quite the equal, in fighting powers, of the black bass of these waters.

29. *Lucius lucius*. Pickerel. Common. Young specimens up to 40 centimeters in length were brought in daily with the minnow seine. They were evidently preying on the various small fishes of the shallow water.

30. *Pomoxis sparoides*. Crappie; Calico Bass. This is a common form in Lake Pepin; the names commonly applied to it here indicate that the fishermen do not distinguish between the two forms of *Pomoxis*. As far as my observations go, however, *P. annularis* does not occur in Lake Pepin.

31. *Ambloplites rupestris*. Rock Bass. Very common. The young are extraordinarily numerous along shore.

32. *Apomotis cyanellus*. A few young specimens seem to belong to this species.

33. *Lepomis pallidus*. Sunfish. Blue Gill. Common. A number of specimens occurred daily in the large seine. They are not marketed, neither are any other of the Centrarchidae.

34. *Micropterus dolomieu*. Black Bass; Small-mouthed Black Bass.

35. *Micropterus salmoides*. Black Bass; Large-mouthed Black Bass.

Both these forms, contrary to general belief, are abundant in Lake Pepin, which seems to offer very favorable breeding grounds for them. Young specimens occur along the shore in such abundance as to be a nuisance in fishing with the minnow seine. Large specimens came in regularly in the big seine, and with the Baird seine as many as fifty were brought in at one haul. It is said that in former years they were caught in large numbers with the hook. This is no longer the case, a catch by hook and line being a rare occurrence. Those fishing for sport are inclined to ascribe this to the commercial seining operations, but with this opinion I am forced to differ. The shores of Lake Pepin are of such extent and character that seining operations can affect only a small part of them. Actual seining, during the two years I was present, was confined practically to the outfit at Rest Island. This seine was hauled over the best beach for this purpose on the lake, but a beach entirely too much exposed to the storm winds to be a suitable place for spawning grounds. What differences there may actually exist between the present and the past as to the readiness of bass in taking the hook must be ascribed to other causes.

I am inclined to ascribe the present indifference of these fish to the line, to the abundance of live food available for them, a cause which I believe to operate similarly on other species in this and other lakes. This, together with the abundance of plankton, the primary source of fish food, probably also makes Lake Pepin so favorable a breeding ground for these and other fishes.

Nor can the fact that game fish are seldom hooked in the lake be considered a calamity; much rather a blessing, from the sportsman's point of view. About four or five miles below Wabasha, Minnesota, and about six or seven miles below Lake Pepin, among the numerous wing dams of the Mississippi River, is to be found some of the finest fishing in the United States, the catch being chiefly small-mouthed and large-mouthed

black bass, walleye and white bass, with smaller numbers of most of the minor game fishes of the region. Considering the comparatively limited area of water, the catch is very heavy, yet the supply keeps up remarkably well. It is evident that for these fishing grounds the spawning grounds of Lake Pepin are a constant source of supply, a source that will always remain inexhaustible, except conditions be changed very materially in the future.

36. *Stizostedion vitreum*. Walleye. A fairly common form here, often of good size. Like all the other game fishes of the lake, it does not readily take the hook here. Several miles below, in the Mississippi, it is caught in great numbers.

37. *Stizostedion canadense griseum*. Sand Pike. A common fish, and one of the few that is readily hooked. It is of rare occurrence in the nets.

38. *Perca flavescens*. Perch. The yellow perch is a rare fish in Lake Pepin. Only a few were caught in our small seines, and much fishing with hook and line failed to produce any.

39. *Percina caprodes*. Log Perch. The log perch is the most numerous of shallow water fishes in Lake Pepin. Most of the specimens are 8 centimeters or less in length; a few of 15 to 16 centimeters were taken. The specimens are all somewhat intermediate between typical *caprodes* and the variety *zebra* of Agassiz. They all have a naked nape, but the fins barred as in the typical form. Depth five and one-half in length.

40. *Hadropterus guentheri*. Only five specimens occur in our collections. Outside of the log perch and Johnny darter, darters are uncommon in the lake.

41. *Boleosoma nigrum*. Of constant occurrence together with *Percina*, but not quite so numerous as the latter.

42. *Roccus chrysops*. White Bass. A common form, almost daily seen in our seines, although always in very small numbers. Many young specimens were caught along shore with the minnow seine. Of all the game fishes of Lake Pepin, this one most readily takes the hook.

43. *Aplodinotus grunniens*. Sheephead; White Perch. This beautiful fish is abundant in Lake Pepin, specimens occurring up to twenty-five pounds in weight. The peculiar sound it produces is quite a characteristic part of every twilight boat ride. Many females with ripe spawn occur by June 15. The fish is marketed in large quantities, though it is decidedly mediocre in quality.

44. *Lota maculosa*. "Eelpout." I spent some time before discovering what the local fishermen designated as an "eelpout," the only name by which they know this fish. The majority seemed to think it a stage in the development of the eel. It is seemingly not a common form in Lake Pepin; we captured only a single specimen in our small Baird seine. It is said to have been formerly caught in large numbers on set lines, and to have been marketed at a good price.

ANATOMICAL AND HISTOLOGICAL STUDIES OF THE DIGESTIVE CANAL OF CIMBEX AMERICANA LEACH.

HENRY H. P. SEVERIN AND HARRY C. M. SEVERIN.

(With Plates I-IV)

The digestive canal of various Hymenoptera has been described by many authors, viz., Swammerdam (27), Réaumur (22), Treviranus¹, Brandt and Ratzeburg (7), Burmeister (8), Newport (20), Dufour (11), and more recently by Schiemenz (23) and Bordas (2, 3, 5, 6). A few of these authors have extended their investigations to different species of the family Tenthredinidae. Burmeister has described the alimentary canal of *Tenthredo nigra*. Newport has worked on the anatomy of *Athalia centifoliae*. He described and figured the digestive canal of this species and did some work on the histological structure. Dufour (11) described and figured the anatomical relations of the digestive organ of a number of species of Tenthredinidae. More recently, Bordas (2, 3, 5, 6) investigated a number of species belonging to this family. His researches on the histological structure of the digestive canal were extended to several genera belonging to various families of Hymenoptera often not closely related.

The material for the present research was obtained in and near the city of Milwaukee, Wisconsin. The insects were all collected during June and the early part of July, usually upon the peach-leaved willow (*Salix amygdaloides* Anders.) and the long-leaved willow (*Salix longifolia* Muhl.).

¹ Paper not accessible.

The males were usually found basking in the hot sunshine upon the leaves of these willows. The females were often taken while working their saws under the epidermis of the lower surface of the leaves, where they deposited their eggs on one or both sides of the mid-rib. Sometimes, however, the females were found girdling the branches with their strong mandibles.

Methods.

For general dissection, the specimens were prepared by injecting 95 per cent alcohol into them with a hypodermic syringe. For histological purposes, the dorsal integument was removed and they were then thrown into the preserving fluid. Several methods of fixation were used, viz., Flemming's weak and strong solutions, Perenyi's fluid and a saturated aqueous solution of corrosive sublimate. In the last-named method, the insects were either killed in hot water, to which, after a few seconds, an equal amount of a saturated aqueous solution of sublimate was added, or the insects, after being killed in hot water, were thrown directly into a hot saturated solution of sublimate. Here the insects were allowed to remain from twenty to forty minutes. They were then thoroughly washed in running water, and finally preserved by placing them in 50, 70 and 95 per cent alcohol consecutively. After this, the different parts of the internal organs were dissected out and carried through absolute alcohol and xylol, infiltrated with 52° paraffin and finally sectioned, the sections being from 4 to 12 μ in thickness. The stains, Flemming's safranin, gentian violet and orange G, and haematoxylin followed by eosin, gave most excellent results.

Anatomical Structure of the Digestive Canal.

The pharynx (Fig. 3, *ph*), which lies wholly within the head, is dorsiventrally flattened, being a little broader and more compressed at the anterior than at the posterior end. Dorsally, the pharynx has a thick layer of circular muscles; ventrally, there is a brown chitinous plate, which ends posteriorly in two prolongations (Fig. 1, *pl*). The pharynx, in pass-

ing through the head, runs in a posterior and slightly dorsal direction, until a position ventral to the brain is reached; here it passes over into the oesophagus, which, instead of continuing in the same direction as the pharynx, makes an angle with it and continues parallel to the long axis of the body.

At its beginning, the oesophagus is as wide as the pharynx, but it gradually narrows and passes as a thin tube straight through the head and thorax. At the anterior end of the abdomen, the oesophagus dilates to form the crop, which, when distended, extends back as far as the sixth abdominal segment (Fig. 2, c). When, however, the crop is not distended, its wall is folded irregularly and, from an external view, is hardly distinguishable from the oesophagus.

The gizzard is in the form of a short cylindrical tube; anteriorly it is pushed into the crop, while posteriorly it is telescoped far into the mid-intestine. From an external view, the only portion visible is a small, slightly curved intermediate piece (Fig. 2, g).

The mid-intestine is a straight tube which is widest at the middle third of its length. This region of the alimentary canal presents a series of circular folds, which are perpendicular to its long axis (Fig. 2, *md*). A microscopical examination of the outer wall shows that there are a number of parallel longitudinal muscles, which send branches into the folds. At the anterior end of the mid-intestine these muscles leave it and attach to the gizzard; at its posterior end they pass backward between the Malpighian tubules, and some continue as the external longitudinal muscles of the ileum.

The Malpighian tubules, ninety to one hundred in number, enter the mid-intestine near its posterior end. A little distance back of their entrance is a constriction, which marks the boundary of the mid-intestine and hind-intestine. Bordas (3) claims that, "Chez les Tenthredinidae (*Emphytus*), les tubes de Malpighi, en nombre très restreint, de 20 à 25, sont insérés sur une seule rangée circulaire, un peu arrière de la portion retrecie du tube digestif, située entre l'intestine terminal." According to Bordas, therefore, the Malpighian tubules of *Emphytus*, a Tenthredinida, must enter the hind-intestine, be-

cause they are inserted posterior to the constriction. We find in *Cimbex americana*, also a Tenthredinida, that the Malpighian tubules enter the mid-intestine at a region anterior to the constriction (Fig. 13, *mlp*).

The next division of the alimentary canal, the hind-intestine, is clearly separable into an anterior convoluted portion, the ileum (Fig. 2, *il*), and a smooth, thicker posterior part, the rectum (Fig. 2, *r*). The ileum is bent once or twice upon itself; the rectum passes as a straight tube through the rest of the abdomen. On the wall of the rectum, and lying parallel with it, are six white elliptical thickenings, the rectal glands (Fig. 2, *rg*). Three of these are situated a little anterior to the other three, which alternate with them.

Histological Structure of the Alimentary Canal.

FORE-INTESTINE.

Oesophagus:—A study of a series of cross sections through the oesophagus shows that at the beginning of this part of the fore-intestine there are a large dorsal and a few smaller lateral and ventral folds (Fig. 4). Passing backward, this large dorsal fold decreases gradually in size, but as it becomes smaller, the lateral and ventral ones become more numerous. In the posterior head region the dorsal fold cannot be distinguished, but here the folds are very irregular in shape and almost fill the lumen. Internally, the lumen is bounded by a hyaline chitinous intima, which is thickest immediately posterior to the pharynx. Throughout the rest of the oesophagus it varies but little in thickness. The chitinous intima rests upon an epithelial layer which, at the anterior end of the oesophagus, is represented by cells with cell boundaries usually indiscernible. Here and there between these epithelial cells are multinucleated glands, which are usually found within the folds (Fig. 4, *gla*). The nuclei of these glands are larger than those of the epithelium. Usually one or more canals can be traced from these glands, passing through the chitinous intima and opening into the lumen of the oesophagus (Fig. 4, *pc*). In the posterior head region and throughout the rest of the

oesophagus we find an epithelial layer, which is represented chiefly by ovoid nuclei, between which no cell boundaries are discernible. The multinucleated glands and their canals have here entirely disappeared. External to this epithelial layer lie the longitudinal muscles, and outside of these, the circular. The circular muscles at the anterior end of the oesophagus are from three to four layers in thickness, but in the thoracic region there are rarely more than two layers. In the thoracic region the circular muscles do not always encircle the oesophagus at right angles to its long axis, but often run obliquely. The circular muscles are covered externally by a peritoneal membrane.

Bordas (3) has extended his researches to several genera of Hymenoptera; he finds that families which are not closely related—Bombinae, Sphecinae, Vespinae, Polistinae, etc.—show a great similarity in the structure of the alimentary canal. His results for all these being nearly the same, he takes as a type of his description *Vespa crabo*. For this species he describes (p. 272) the muscular layers of the oesophagus and crop as follows:—"Ils comprennent, en allant de l'intérieur vers l'extérieur, une couche chitineuse, incolore, généralement peu épaisse et reposant sur une très mince *couche cellulaire*; vient ensuite une *enveloppe musculaire* formée par des fibres circulaires, abondantes surtout à la base des sinuosités rentrantes; enfin, extérieurement existe une assise constituée par des faisceaux musculaires longitudinaux." In a later work (5, p. 361), he again describes the muscular layers of *Vespa crabo* as follows:—

"Man findet demnach auf einem zur Achse senkrechten Schnitte, von aussen nach innen gehend, die folgenden verschiedenen Schichten:

1. Eine sehr feine peritoneale Membran, die sich über den Oesophagus, Kropf und ganzen Darm ausdehnt (*mp*).

2. Eine erste Lage von Ringmuskelfibrillen, die durch die fast regelmässige Überlagerung von zwei oder drei Schichten, welche überall dieselbe Dicke besitzen, gebildet wird (*fc*).

3. Längsmuskeln (*fl*). Diese letzteren sind in Bündeln von verschiedener Zahl angeordnet. Die auf dem Querschnitt

dreiseitigen in den Oesophagealraum vorragenden Bündel bilden so eine Art innerer Falten. Zwischen diesen letzteren nehmen andere Längsmuskelbündel die Räume verminderten Umfanges in bezug auf die vorigen Gruppen ein. Die Zahl der Longitudinalschichten ist sehr verschieden. Das, was diese Muskulatur charakterisiert, ist ihre Anordnung, die Unregelmässigkeit ihrer Dicke und vor allem ihre Falten."

In the first-mentioned paper (3), Bordas claims that external longitudinal and inner circular muscles are present, the latter being especially abundant at the base of the sinuous folds; in the second paper (5), however, he does not mention these external longitudinal muscles, but claims that internal longitudinal muscles are present. He figures, in his later work (5), a cross section of the oesophagus and shows the circular muscles far removed from the folds, but at those regions where the inner longitudinal muscles are arranged in groups they are present even at the base of the folds.

Crop.—The histological structure of the walls of the crop tends to prove that it is simply a dilation of the posterior end of the oesophagus. The internal folds of the walls are somewhat larger and more numerous in the crop than in the oesophagus (Fig. 7, *fc*). We find no distinction in the epithelium between the oesophagus and crop; the cells of both parts being represented chiefly by ovoid nuclei, between which no cell boundaries are discernible. Toward the posterior region of the crop, some of the internal longitudinal muscles penetrate the circular layers and continue as external longitudinal muscles, which attach to the chitinous intima at various parts of the gizzard (Fig. 7, *lmc*).

Gizzard.—A longitudinal section through the proventriculus shows that it may be divided into three parts:—(1) an anterior portion invaginated into the crop [*"Verschlusskopf"* of Schiemenz (23)]; (2) a central part, easily seen in a dissection (Fig. 2, *g*) [*"Hals"* of Schiemenz (23) and *"pedunculus"* of Bordas (5)]; and (3) a posterior portion telescoped far into the mid-intestine [*"Zapfen"* of Schiemenz (23) and *"appendice vermiforme"* of Bordas (5)].

The histological change, at the place where the crop passes over into the gizzard, is abrupt. At this region the large, narrow folds of the crop gradually become flattened and finally form an almost unfolded chitinized epithelium, which continues over into the unfolded chitinized epithelium of the gizzard. In the former, the thin, hyaline chitinous layer with its numerous blunt projections passes over, in the gizzard, into a thick intima which has numerous long, backward-pointing bristles (Fig. 7, *b*). The cellular change between these two parts of the fore-intestine is more abrupt. The ovoid nuclei of the epithelial cells of the crop assume a direction parallel to the almost unfolded chitin; these cells are then replaced in the gizzard by cubical cells with large spherical nuclei.

The most striking feature in a longitudinal section of the gizzard is the thickness of the muscle layers and their attachments. Externally, some of the longitudinal muscles can be traced from the mid-intestine directly to the gizzard; others, however, like some of the longitudinal muscles of the crop, attach to the chitinous intima of the gizzard (Fig. 7, *lm. md*). Numerous branches from these external longitudinal muscles, and sometimes an entire fiber, penetrate the circular and inner longitudinal layers (where the latter are present), spread out in a somewhat fan-shaped manner just before reaching the epithelial cells, and finally attach to the chitinous intima. This peculiar branching continues from the beginning of the mid-intestine as far as the external longitudinal muscles extend anteriorly (Fig. 7, *blm*). Some of the external longitudinal muscles attach to the chitinous intima near the beginning of the gizzard. The circular muscles just internal to these longitudinal ones are exceedingly thick; they attain their maximum thickness near the beginning of the "pedunculus" and disappear near the anterior end of the appendix. Following these circular layers within are the inner longitudinal muscles, which attach anteriorly to the chitin of the gizzard (Fig. 7, *ilm*); posteriorly, some of these muscles attach to the chitinous layer at the anterior end of the "pedunculus," while others can be traced to the posterior end of it.

A transverse section through the anterior part of the gizzard shows that the strongly chitinized walls are thrown into four large folds or valves (Fig. 5). From the thick chitinous intima, numerous long yellowish bristles project into the lumen (Fig. 5, *b*); these undoubtedly, as in the honey-bee, serve as a sort of filter. Next outside of the chitinous intima is an epithelium, which is made up of cubical cells containing a large spherical nucleus. Within the four irregular folds or valves is the next layer, the internal longitudinal muscles. Some of the branches from the external longitudinal muscles pass into these folds and attach to the chitinous intima; others attach to the chitin between the folds (Fig. 5, *blm*). Surrounding the four valves are the greatly developed circular muscle layers, which serve to contract the four powerful folds. Outside of the circular muscles are the external longitudinal muscles, which are covered externally by a thin peritoneal membrane.

A study of a series of transverse sections through the gizzard shows that these four valves do not extend throughout its entire length; secondary folds make their appearance, which, posteriorly, become gradually larger and larger, until they are equal in size to the primary ones. The bristles, which project from the chitinous intima into the lumen of the anterior part of the gizzard, have disappeared near the beginning of the "pedunculus." Figure 6, a transverse section through the posterior end of the "pedunculus," shows that at this region the folds have become nearly equal in size but are not regular in shape. Within the folds, as well as between them, branches from the external longitudinal muscles are found to attach to the chitinous intima.

The appendix [*"Zapfen"* of Schiemenz (23) or *"appendix vermiforme"* of Bordas (5)], a continuation of the "pedunculus," is a cylindrical tube with irregular longitudinal folds, and is telescoped far into the lumen of the mid-intestine. A longitudinal section through it shows that the chitinous intima, which is a direct continuation of the somewhat thicker chitin of the "pedunculus," continues back as a very irregular layer with numerous large, sharply-pointed projections to the pos-

terior opening of the appendix (Fig. 7, *app*). The chitin becomes gradually thinner as it bends upon itself, and then extends anteriorly as an outer chitinous intima of almost uniform thickness. This outer intima is covered with numerous blunt projections, which do not disappear until near the place of union with the mid-intestine. Here it gradually becomes thinner, and finally is replaced by the striated intima of the mid-intestine (Fig. 7, *sm*). In most of the longitudinal sections, the outer chitinous intima was separated from the underlying cellular layer, a peculiarity which Bordas (5) has also noticed. In the tubular part of the appendix, the epithelial cells are very much flattened, with cell boundaries not discernible. Each cell contains a single ovoid nucleus which has its long axis parallel to the chitin. These epithelial cells continue as such around the posterior opening of the appendix, but gradually increase in size anteriorly and finally are replaced by the long, cylindrical epithelial cells of the mid-intestine. Between the two epithelial layers of the appendix is a narrow space which is filled with connective tissue (Fig. 7, *cct*). At the anterior part of the appendix, numerous branches from the external longitudinal muscles of the gizzard attach to the inner chitinous intima.

Bordas (5) determined experimentally the function of the appendix in *Vespa*. His results, which are of special interest, are as follows:—"Das Zurücktreten der Nahrung von hinten nach vorne zu verhindern, d. h. vom Mitteldarm während dessen peristaltischen Bewegungen zum Kropfe, fällt im besonderen dem wurmförmigen Appendix zu, der der Darmklappe bei den *Apidae* und anderen Insekten entspricht. Ich habe tatsächlich experimentell festgestellt, dass der Rücktritt der Nahrung unmöglich ist. Es genügt hierfür eine gefärbte Flüssigkeit in den Mitteldarm von *Vespa* zu injizieren. Übt man dann einen Druck von hinten nach vorne aus, bemerkt man, dass nicht ein Tropfen der Flüssigkeit in dem wurmförmigen Appendix gelangt, und dass sie sich zwischen seinen äusseren Wänden und denen des Mitteldarmes anhäuft. Überdies ist es leicht einzusehen, dass, je grösser der auf die Flüssigkeit ausgeübte Druck ist, diese umso mehr die Wände des

Appendix zusammenpresst und seinen Verschluss um so hermetischer macht, der so das Eindringen jeder Substanz, sei sie flüssig oder nicht, durch die Endöffnung verhindert."

MID-INTESTINE.

Throughout the mid-intestine, the transversely folded wall is nearly uniform in structure and consists of a number of layers which are, passing from within outward:—(1) a layer of cylindrical epithelial cells with a striated intima bounding the lumen and groups of regenerating cells ["*Drüsen-Crypten*" of Frenzel (13), "*Drüsen*" or "*Crypten*" of Faussek (12), "*germinal buds*" of Miall and Denny (18) and "*nidii*" of Needham (19)] scattered among their bases; (2) a *membrana propria*, or basement membrane; (3) a layer of circular muscles; (4) a layer of longitudinal muscles; and (5) a peritoneal membrane.

A study of the free edge of the epithelial cells shows the presence of secretory processes, which are formed by strangulation. The secretory processes are of two kinds; the larger generally represents a large part of an epithelial cell and sometimes includes its nucleus, while the smaller is much more numerous and never contains a nucleus. These larger secretory processes are usually pyriform in shape and protrude far above the striated intima of the epithelial cells. In Figure 11, the large secretory process (*lsp*) has the appearance of having been crowded out by the compression of the adjacent cells. The smaller kind of secretory processes are stalked granular globules in direct continuation with the protoplasm of the cell from which they have originated (Fig. 11, *ssp*). We could observe only one way in which the secretory processes became liberated into the lumen of the intestine; they were simply constricted off from the cell, and were then free to circulate within the intestine (Fig. 11, *sp*).

The epithelial layer consists of two markedly different parts; the elongated, cylindrical epithelial cells, and little groups of regenerating cells lying at their bases. On account of the regular transverse folds of the mid-intestine, the epithelial cells

are of various forms and dimensions according to their position. They are usually elongated, and more or less rounded toward their free ends. Toward the basal end of the cells, the cytoplasm shows in many places a distinct longitudinal striation (Figs. 10 and 11, *ls*), more noticeable in some sections than in others; the cells, especially at the free ends, are partially filled with globules, which stain a bluish color with haematoxylin. These globules are not uniformly distributed throughout the cell; they usually decrease in number toward the basal end. Here and there, however, there are cells in which even the basal portion is crowded with globules. Each epithelial cell contains a single spherical or ovoid nucleus, which contains a small number (one to six) of nucleoli. Here and there, young cells, each containing a small nucleus, are wedged in between older ones. Clustered together just inside of the basement membrane, between some of the epithelial cells, are small groups of from one to six or more regenerating cells (Fig. 10, *rc*). We were unable to find any globules in the regenerating cells, but the cytoplasm stains more lightly than in the cells of the regular epithelium. The nuclei of these regenerating cells are smaller and stain more deeply with haematoxylin than do the nuclei of the long, cylindrical epithelial cells.

The basement membrane, upon which rests the epithelium, is covered with a layer of circular muscles; these are abundant, especially at the inner end of the transverse folds. Outside of the circular muscles are the longitudinal, which, in an external view, appear as longitudinal fibers running parallel to one another. From these, branches are given off, which pass into the transverse folds of the mid-intestine. In a longitudinal section we were unable to ascertain with certainty the attachment of these branches. The longitudinal muscles extend posteriorly between the Malpighian tubules, and some continue as the external longitudinal muscles of the ileum.

Malpighian Tubules:—A transverse section through the digestive canal at the entrance of the Malpighian tubules shows that each opens separately, and that they do not all enter the mid-intestine in the same plane. Anteriorly and posteriorly to their entrance, the epithelial cells of the mid-intestine be-

come somewhat smaller, and gradually in the Malpighian tubule assume a more or less flattened shape. Each cell contains a single ovoid nucleus, which has its long axis parallel to the corresponding axis of the tubule. The striated intima is continuous from the wall of the mid-intestine over into the Malpighian tubules whose entire inner surface it covers (Figs. 13 and 14, *sm*).

A more minute study of the Malpighian tubules shows that in a transverse section the cells are generally six in number and arranged in a circle. Each cell is somewhat longer than wide, with its inner surface rounded. The striated intima which rests upon this rounded surface bounds a more or less sinuous lumen (Fig. 9, *sm*). A peculiarity noticeable in the cells of some tubules is the presence of bluish staining globules, which, in general appearance, resemble those in the epithelial cells of the mid-intestine (Fig. 9, *gl*). The cells rest upon a basement membrane, external to which is a thin peritoneal membrane (Fig. 9, *np*).

HIND-INTESTINE.

Ileum:—Posterior to the entrance of the Malpighian tubules, the mid-intestine continues back a short distance and then passes over into the hind-intestine. Histologically, we find an abrupt change; the long, cylindrical epithelial cells of the mid-intestine are replaced at the beginning of the hind-intestine by smaller cells with relatively small nuclei. The striated intima which covers the inner surface of the entire mid-intestine and Malpighian tubules is replaced in the ileum by a hyaline chitinous intima (Fig. 8).

Pyloric valve:—A longitudinal section through the anterior part of the ileum shows the presence of the so-called pyloric valve (Fig. 13, *pv*) [*"valvule rectale"* of Balbiani,² *"sphincter de l'intestine grele"* of Van Gehuchten (15)]. The lumen of the canal decreases abruptly in size immediately posterior to the boundary between the mid-intestine and hind-intestine. Numerous conical, backward-pointing spines project from the

²Quoted from Van Gehuchten's paper.

thin chitinous intima into the lumen of the pyloric valve. The circular muscles are greatly developed, and, according to Van Gehuchten, "peuvent être considérés comme un appareil d'occlusion, destiné à empêcher les matières alimentaires, soit de passer trop vite dans l'intestine terminal, soit de revenir du gros intestine dans le ventricule chylique."

A transverse section through the pyloric valve shows that the epithelial layer, which is lined with a chitinous intima, is thrown into a number of broad folds (15 to 18). The epithelial cells of these folds are little longer than broad, while in the space between the folds they are more flattened. The long axis of the ovoid nuclei is usually at right angles to the chitinous intima. External to the thick circular muscles are the longitudinal, which can be traced from the mid-intestine, between the Malpighian tubules, over to the ileum. These are covered externally by the peritoneal membrane.

Posterior to the pyloric valve, the folds of the ileum become less numerous and very irregular in shape. The conical spines which project from the chitinous intima into the lumen of the pyloric valve are replaced by blunt projections. The cells contain a slightly granular cytoplasm, which, in places, shows a distinct longitudinal striation. The nucleus, usually ovoid in shape, takes up the larger part of the cell (Fig. 12). The circular muscles, after their temporary thickening at the pyloric valve, pass over into a thin layer. From the external longitudinal muscles, branches are given off which enter the folds of the ileum and attach to the chitinous intima. The longitudinal muscles are covered by a thin, delicate peritoneal membrane.

The ileum is invaginated into the rectum, as is shown in a longitudinal section through these two parts (Fig. 15, *il*). At this region, the ileum attains a somewhat better development of the circular muscles, and the folds almost fill up the entire lumen. Evidently this great development of the circular muscles serves, when they contract, to retain the nourishment within the ileum. According to Newport (20), the ileum of *Athalia centifoliae* also ends in a valve. We propose to call this valve the *posterior iliac valve*, to distinguish it from the pyloric valve

[“*valvule rectale*” of Balbiani, “*sphincter de l’intestine grele*” of Van Gehuchten (15)] of the anterior ileum. This valve prevents the material in the hind-intestine from passing back into the ileum after it has once entered the rectum.

Rectum:—The rectum shows a marked histological difference from the ileum. Near the boundary between these two divisions of the hind-intestine, the cells of the ileum become smaller and pass abruptly into the much reduced cells of the rectum. The walls of the entire rectum, with the exception of the rectal glands, are thrown up into irregular internal folds. The cells, with cell boundaries indiscernible, are represented chiefly by their nuclei. These cells and the folded nature of the rectum show a marked similarity to the structure of the oesophagus and crop.

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EXPLANATION OF PLATE I.

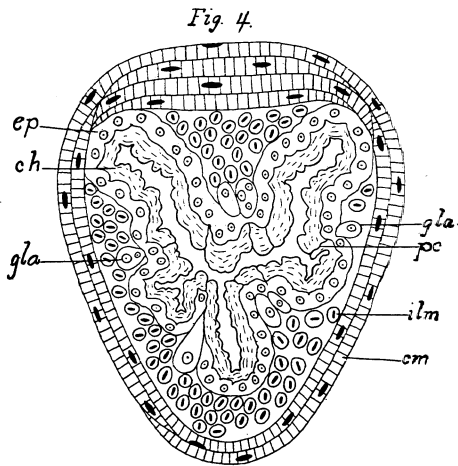
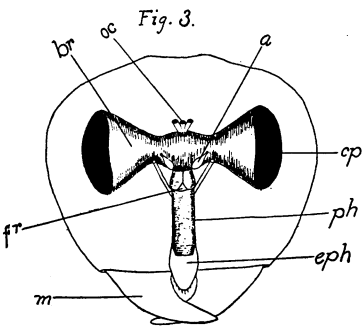
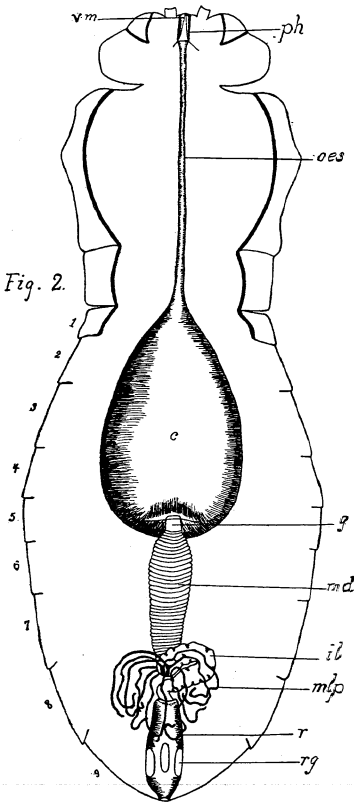
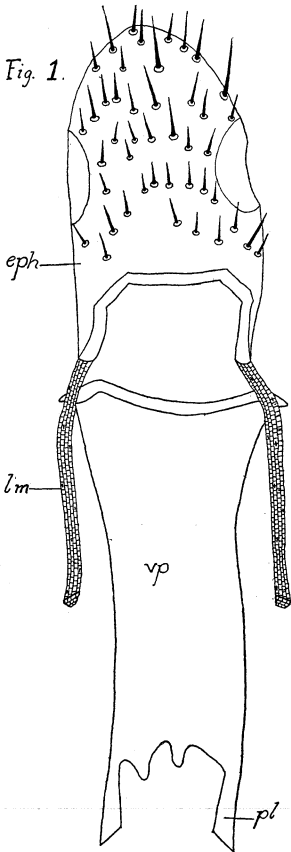
All figures except general dissections were drawn with a camera lucida. Magnifications in diameters are given after the explanation of each figure.

Fig. 1. Epipharynx, and pharynx with dorsal wall not shown:—*vp*, ventral plate of pharynx; *lm*, bundles of longitudinal muscles which are inserted at the base of each side of the epipharynx; *pl*, prolongations of posterior part of pharynx. (x 40.)

Fig. 2. Dissection showing dorsal view of alimentary canal:—*ph*, pharynx; *vm*, vertical muscles; *oes*, oesophagus; *c*, crop; *g*, gizzard; *md*, mid-intestine; *il*, ileum; *mlp*, Malpighian tubules; *r*, rectum; *rg*, rectal glands. (x 4.)

Fig. 3. Cephalic integument of head removed, showing the pharynx and brain:—*m*, mandible; *eph*, epipharynx; *ph*, pharynx; *br*, brain; *cp*, compound eye; *oc*, three ocelli; *fr*, frontal ganglion; *a*, antennal lobe. (x 6.)

Fig. 4. Cross section of oesophagus near pharynx, showing the large dorsal fold:—*ch*, chitinous intima; *ep*, epithelium; *ilm*, longitudinal muscles; *cm*, circular muscles; *gla*, multinucleated gland; *pc*, pores which penetrate the chitinous intima. (x 216.)



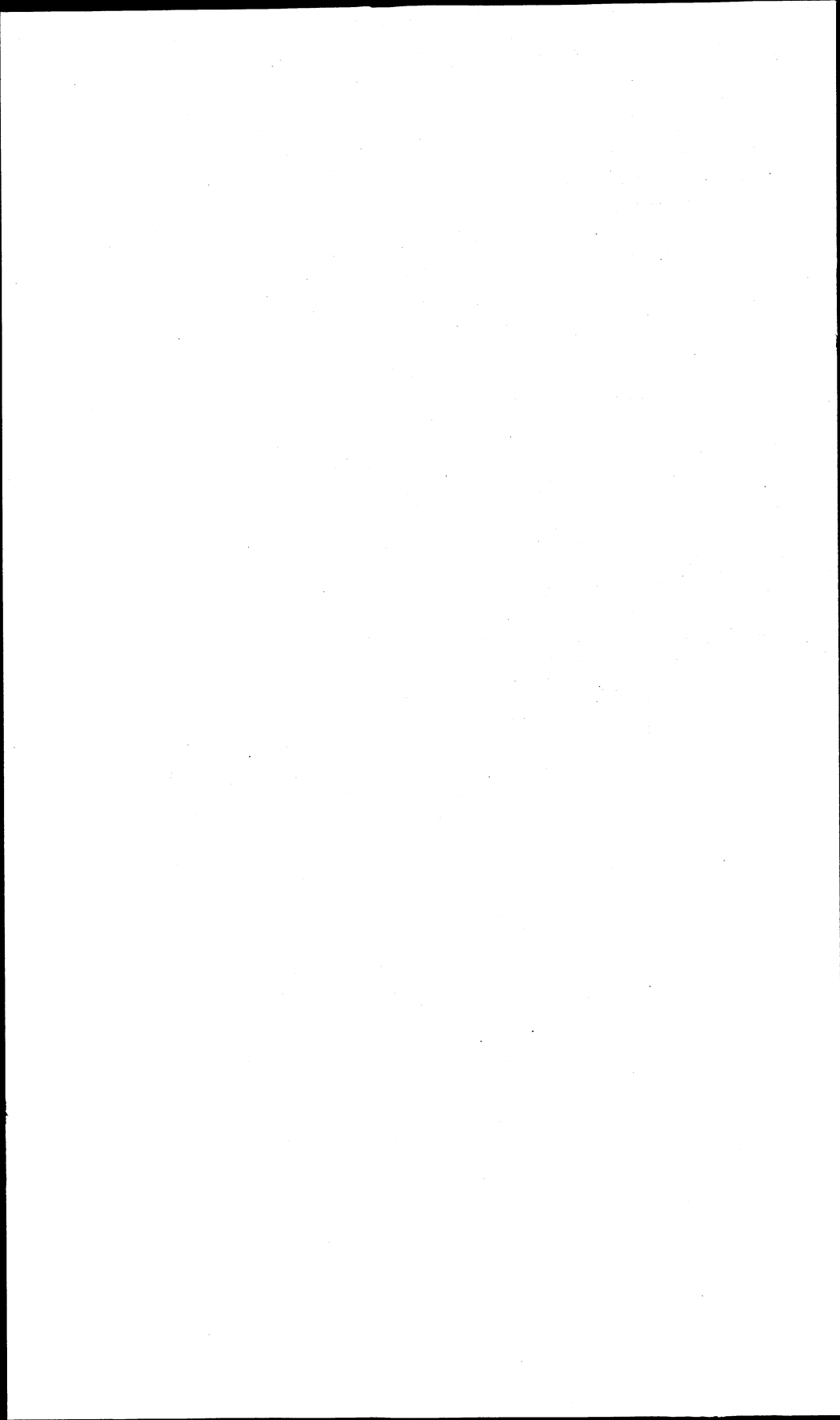


PLATE II.

EXPLANATION OF PLATE II.

Fig. 5. Transverse section through the anterior part of the gizzard, showing four large folds:—*b*, bristles; *ch*, chitinous intima; *ep*, epithelium; *ilm*, inner longitudinal muscles; *cm*, circular muscles; *elm*, external longitudinal muscles; *blm*, branch of the external muscles; the peritoneal membrane is not shown. (x 150.)

Fig. 6. Cross section through the posterior end of the pedunculus:—Lettering as in Figure 5. (x 150.)

Fig. 7. Longitudinal section through the gizzard; the anterior part is invaginated into the crop (*c*), and the posterior portion is telescoped into the mid-intestine (*md*):—*lmc*, external longitudinal muscles which pass over from the crop and attach to the chitinous intima of the gizzard; *fc*, folds of the crop; *b*, bristles; *ch*, chitinous intima; *nep*, nucleus of epithelial cell; *cm*, circular muscles; *ilm*, inner longitudinal muscles; *elm*, external longitudinal muscles; *blm*, branches of external longitudinal muscles—occasionally an entire muscle fiber penetrates the circular muscles and attaches to the chitinous intima; *p*, peritoneal membrane; *lm md*, longitudinal muscles which pass over from the mid-intestine and attach to the chitinous intima of the gizzard; *bo*, boundary between gizzard and mid-intestine; *sm*, striated intima of epithelial cells of mid-intestine; *app*, appendix; *cct*, connective tissue. (x 66.)

Fig. 8. Part of a longitudinal section, showing cellular change between mid-intestine and hind-intestine:—*bo*, boundary between mid-intestine and ileum; *sm*, striated intima of epithelial cells of mid-intestine; *ep md*, epithelial cells of mid-intestine; *ep il*, epithelial cells of ileum; *ic*, chitinous teeth. (x 633.)

Fig. 9. Cross section of Malpighian tubule, showing numerous globules (*gl*):—*nep*, nucleus of epithelial cell; *sm*, striated intima of epithelial cell; *bm*, basement membrane; *np*, nucleus of peritoneal membrane. (x 633.)

Fig. 6.

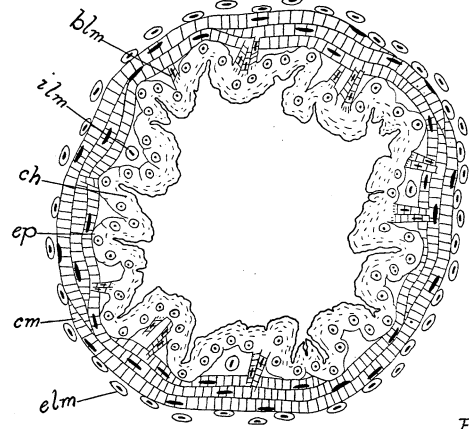


Fig. 8.

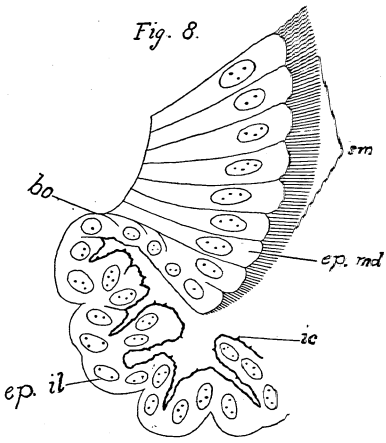


Fig. 5.

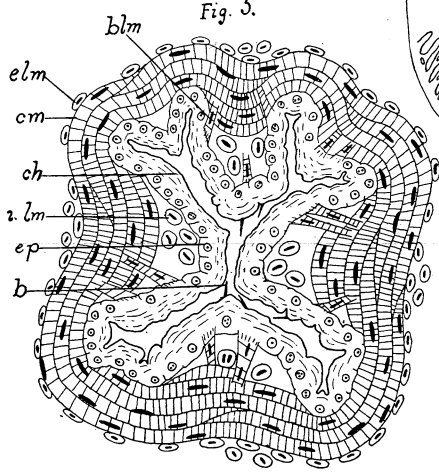


Fig. 7.

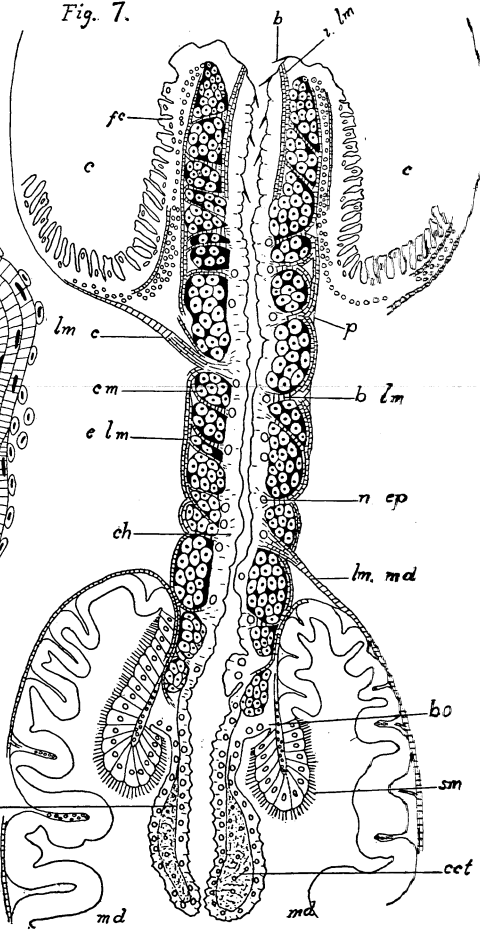


Fig. 9.

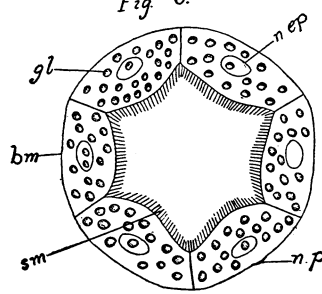


PLATE III.

EXPLANATION OF PLATE III.

Fig. 10. Epithelial cells of mid-intestine, with a regenerating center (*rc*):—*sm*, striated intima; *nep*, nucleus of epithelial cell; *ls*, longitudinal striations of cytoplasm; *bm*, basement membrane. (x 760.)

Fig. 11. Epithelial cells of mid-intestine, with secretory processes:—*lsp*, large secretory process; *ssp*, small secretory process; *sp*, secretory process which has been constricted off from epithelial cell and is free to circulate in the mid-intestine; *sm*, striated intima of epithelial cells; *ls*, longitudinal striations of cytoplasm; *bm*, basement membrane. (x 640.)

Fig. 12. Cells of ileum:—*nep*, nucleus, which takes up the greater part of the cell; *ch*, chitinous intima; the muscular layers are not shown. (x 760.)

Fig. 13. Longitudinal section through mid-intestine and hind-intestine, showing pyloric valve (*pv*):—*bo*, boundary between mid-intestine and hind-intestine; *ep*, epithelium; *sm*, striated intima of epithelial cells of mid-intestine; *mlp*, Malpighian tubule opening into mid-intestine; *elm*, external longitudinal muscles; *cm*, circular muscles; *blm*, branch of external longitudinal muscles; *ch*, chitinous intima. (x 80.)

Fig. 10.

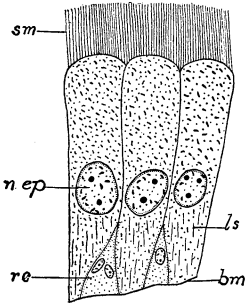


Fig. 11.

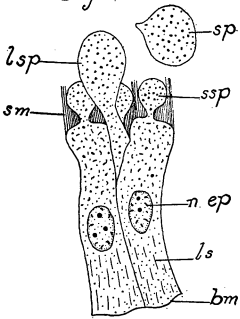


Fig. 12.

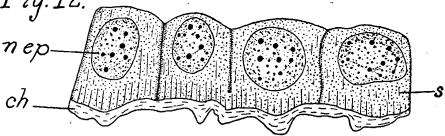


Fig. 13.

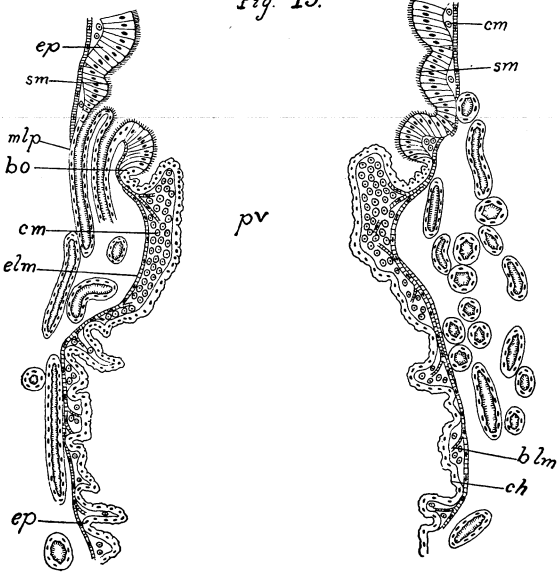
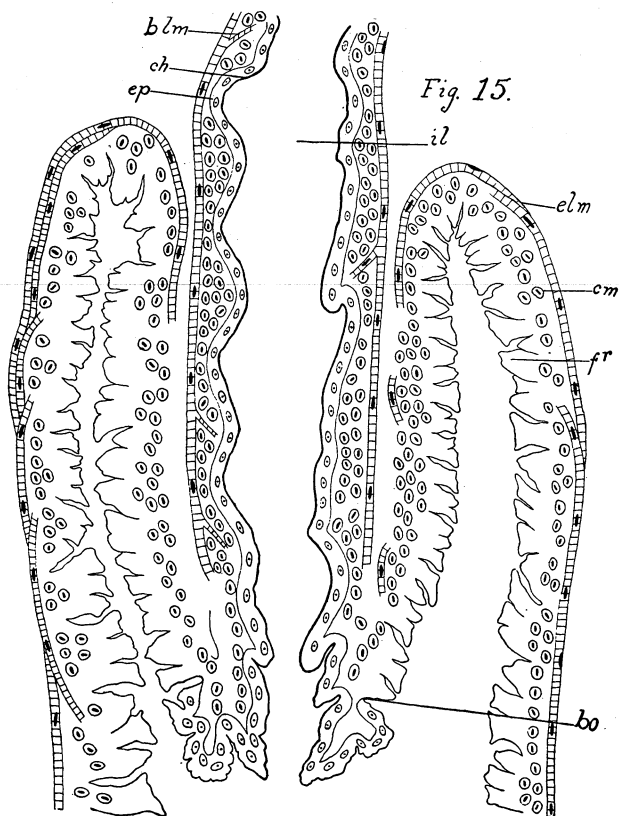
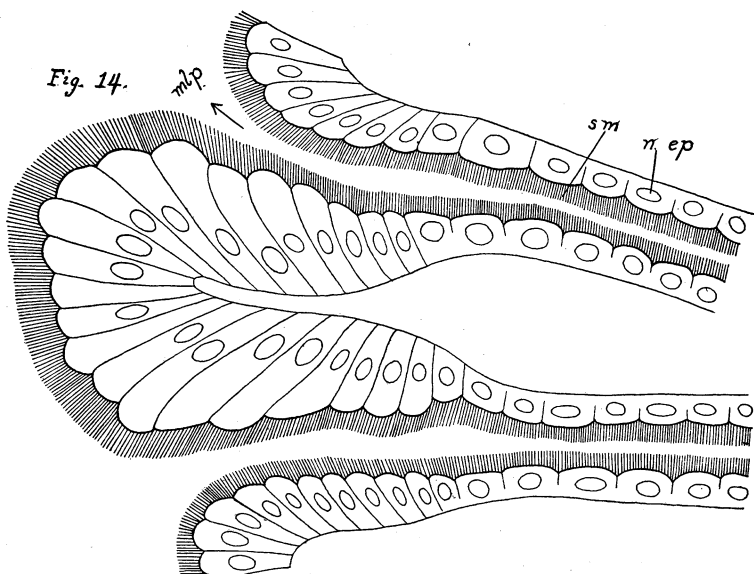


PLATE IV.

EXPLANATION OF PLATE IV.

Fig. 14. Section through two Malpighian tubules at their entrance into the mid-intestine, showing the gradual passing over of the large cylindrical cells of the mid-intestine into the somewhat flattened cells of the Malpighian tubules; *mlp*, opening of Malpighian tubule into mid-intestine; *sm*, striated intima; *nep*, nucleus of epithelial cell. (x 525.)

Fig. 15. Longitudinal section through the posterior iliac valve:—*il*, ileum; *fr*, folds of rectum; *ch*, chitinous intima of ileum; *ep*, epithelium of ileum; *elm*, external longitudinal muscles; *blm*, branch of external longitudinal muscles; *cm*, circular muscles; *bo*, boundary between ileum and rectum. (x 116.)



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Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains.

Figure 1 illustrates the experimental setup. A subject is seated at a table, viewing a video screen. A video camera is positioned above the screen. A light source is positioned to the left of the screen. A target is positioned on the screen. A ruler is placed on the table. A scale bar is shown at the bottom right.

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HABITS OF THE AMERICAN SAW-FLY, *CIMBEX AMERICANA* LEACH, WITH OBSERVATIONS ON ITS EGG-PARASITE, *TRICHOGRAMMA PRETIOSA* RILEY.

HENRY H. P. SEVERIN AND HARRY C. M. SEVERIN.

(With Plate V)

INTRODUCTION.

While collecting the American saw-fly, *Cimbex americana*, for anatomical and histological studies, our attention was drawn to the habits of this insect. The specimens were usually found on two species of willows, the peach-leaved willow, *Salix amygdaloides* Anders., and the long-leaved willow, *Salix longifolia* Muhl. Our observations during the past summer have been confined to Milwaukee County, Wisconsin, where we have had opportunities of watching the daily progress of the insect.

DESCRIPTION OF MALE AND FEMALE SAW-FLY.

As there is a considerable amount of individual variation in both male and female saw-flies, it has seemed advisable to describe both sexes. The head and thorax of the female are shining black. Its abdomen is ovoid in shape and of a steel-blue color, with three, four or five whitish or yellowish markings on each dorso-lateral side. The antennae are short and end in an egg-shaped knob; they are buff-colored, except towards the proximal end where they are somewhat darker. The wings are smoky brown and measure, when expanded,

from forty-five to fifty millimeters. The legs are steel-blue, with the exception of the tarsi, which are pale yellow. The length of the body measures from twenty to twenty-two millimeters.

The male differs so much from the female that it might be taken for a different species. The body is longer and narrower than that of the female, measuring twenty-five millimeters or slightly more in length. Either the abdomen is of a steel-blue color, or the anterior half is bluish-black with the posterior portion reddish-brown; the whitish or yellowish markings which are so conspicuous in the female are wanting. The wings are either smoky brown or semi-transparent and expand from fifty to fifty-five millimeters. One noticeable peculiarity of the male is a transverse oval hole, covered with a white membrane, between the alitrunk and the abdomen; this is hardly perceptible in the other sex.

Individual variation exists in the number of whitish or yellowish markings on each side of the dorso-lateral sides of the abdomen. Harris (3), in describing the female, writes as follows:—"Her hind body is oval and of a steel-blue or deep violet color, with three or four oval, yellowish spots on each side." Comstock (1), in his general description of the American sawfly, writes:—"The female is about three-fourths of an inch long and has a black head and thorax, a steel-blue or purplish abdomen, with four yellowish spots on each side." In order to ascertain the most frequent number of whitish or yellowish markings on each side of the abdomen, we examined seventy-five specimens with the following results:—

8 specimens with three spots on each side of dorso-lateral abdomen.

23 specimens with four spots on each side of dorso-lateral abdomen.

44 specimens with five spots on each side of dorso-lateral abdomen.

Although the number of specimens examined is rather small to draw any definite conclusions from, it appears that the most frequent number of markings on each side of the abdomen is five. Undoubtedly Harris and Comstock failed to ex-

amine a large number of specimens, or they would have noticed this individual variation.

It is worthy of notice that the six-spotted specimens always had one pair of spots each on the fourth, fifth and sixth abdominal segments. Occasionally a specimen was found on which a pair of spots was very faintly indicated on either the third or the seventh segments. On these specimens the spots were usually small, and either circular or oval in outline. Those specimens which were marked with four spots on either side of the abdomen had usually one pair each on the third, fourth, fifth and sixth segments, the markings on the seventh segment usually being absent. Specimens marked with five spots on each side of the abdomen always had them on the third, fourth, fifth, sixth and seventh segments. Those specimens which were marked with eight or ten spots usually have those on the fourth, fifth and sixth segments in the shape of bands, covering the length of the segment and extending to near the dorsal median line of the abdomen.

In the male we also found individual variation in color. Some of the specimens have the entire abdomen of a steel-blue color, while others have the anterior half of the abdomen steel-blue and the posterior portion reddish-brown. We examined fifty males with the following results:—

11 specimens with the entire abdomen steel-blue.

39 specimens with the anterior half of the abdomen steel-blue and the posterior portion reddish-brown.

Although but fifty specimens were examined, it is evident that those males having the abdomen partly steel-blue and partly reddish-brown greatly outnumber those having an entirely steel-blue abdomen.

GENERAL HABITS.

The imago first makes its appearance about the beginning of June and disappears toward the middle of July. Both males and females are more active during the middle of a bright hot day of sunshine, flying about with a loud buzzing noise. As the air grows cooler, however, they become duller in all their movements. The males are usually found with

wings slightly spread apart, basking in the hot sunshine on the leaves at the tips of the highest branches. If a male, for some reason or other, takes to wing, oftentimes one or several other males will also leave their resting place, and, hovering about one another, will rise high into the air, only finally to come down again and take another sun bath on the leaves at the tops of the willow trees. The females are often found busily engaged in depositing their eggs, usually above the lower epidermis of the leaves. Oftentimes, however, they may be found girdling branches with their strong mandibles.

DEPOSITION OF THE EGGS.

The female usually assumes a very characteristic position in the act of oviposition. During the operation, she perches herself upon the margin of the leaf and always with her body in its longitudinal direction. The claws and tarsi of the three legs on one side of the body hold on to the dorsal surface of the leaf, while the margin of the leaf is usually held in the joint between the femur and tibia of these same legs. The claws of the three legs on the other side of the body usually cling to the mid-rib on the ventral surface of the leaf. Being perched in this position, she curls up the abdomen, places the distal end of the scabbard-like sheaths of the saws against the ventral surface of the leaf near its margin and then shoves out the saws obliquely. By an alternating movement of the two saws, one being thrust forward while the other is retracted, she begins to cut an incision above the lower epidermis. Each saw slides up and down in a groove to steady it. This groove is found within the supports of the saw, the pair of supports being united at the base. The insect during the sawing act often lets go and again catches hold with its claws as if to secure a better footing. Farther and farther she passes her ovipositor towards the mid-rib until she has passed it into the leaf its whole length, and then she widens out the cavity. Finally the saws cease to move, and the receptacle is completed. The abdomen contracts a few times, and then a pale-greenish egg appears near the distal end of the saws. The saw sways across the receptacle as the egg is being passed out, and then

a white fluid from the collateral sac is poured over the egg into the receptacle. Usually the insect proceeds in the same manner to deposit a second, a third, or more eggs, always taking care to place but one in each receptacle (Fig. 1, *e*). No difficulty is experienced in observing the operation, the female saw-flies not having the timidity of many other insects and not being easily disturbed while laying their eggs.

The whole process of depositing an egg occupies about one minute. Oftentimes a specimen was observed that made an incision (Figs. 1 and 2, *i*) or almost completed a receptacle (Figs. 1 and 2, *pr*), when the saws struck a side-rib and the sawing ceased. Sometimes there was no side-rib to obstruct the sawing, but for some unknown reason an active female would not complete the receptacle, or, if she did, would not deposit an egg. Specimens which were near the end of their natural life frequently cut a large number of incisions or receptacles without depositing an egg. One specimen, which we observed, cut fourteen incisions in one leaf and thirty incisions in another leaf without either completing a receptacle or depositing an egg. These specimens, when they did finally complete a receptacle and deposit an egg, required more than one minute for the whole process. Table I shows the number of incisions and partially completed receptacles found on one hundred leaves from a single peach-leaved willow and another hundred from a few long-leaved willows.

The receptacle is a little longer than wide (Figs. 1 and 2, *r*). It measures a little less than five millimeters in length and four to four and one-half millimeters across its widest region. Upon the long-leaved willow the receptacle is often smaller and is situated either near or against the mid-rib. Rarely does the receptacle extend beyond the mid-rib, being more often flattened up against it. Upon the peach-leaved willow the mouth of the receptacle (Figs. 1 and 2, *mr*) is about three millimeters from the margin of the leaf, and its base is usually four to five millimeters from the mid-rib except towards the apical end of the leaf where the mouth of the receptacle is somewhat closer to the margin of the leaf and the base often rests against the mid-rib. In both kinds of leaves, the

receptacle is often found between the intervening side ribs (Figs. 1 and 2).

Extending transversely across the basal region of the receptacle is a single elongated, oval egg, with a distinction between dorsal and ventral surfaces indicated by a difference in curvature. The ventral, or convex side of the egg is always nearest the mid-rib, while the dorsal or concave side lies nearest the margin of the leaf (Fig. 1, *e*). The egg, soon after being deposited by the female, measures two and one-half millimeters in length.

The following table shows the number of eggs deposited on the left and right sides of the mid-rib, the total number of eggs deposited, the number of eggs parasitized, the number of eggs hatched, the number of incisions, and lastly the number of partially completed receptacles, of one hundred leaves from a single peach-leaved willow and another hundred from a few long-leaved willows.

TABLE I.

	Peach-leaved willow.	Long-leaved willow.
Eggs deposited on right side of mid-rib.....	207	142
Eggs deposited on left side of mid rib.....	188	138
Total number of eggs on one hundred leaves	395	280
Total number of eggs hatched.....	353	251
Eggs parasitized by <i>Trichogramma pretiosa</i>	5	6
Eggs which failed to hatch from other causes.....	37	23
Incisions.....	109	179
Partially completed receptacles.....	74	53

On many leaves we observed only one egg deposited, but on others as many as six, eight, or even fourteen were found on one or both sides of the mid-rib. An examination of the one hundred leaves of the peach-leaved willow showed that nineteen more eggs were deposited on the right side of the mid-rib than on the left side; whereas, upon the one hundred leaves of the long-leaved willow there were one hundred and forty-two eggs on the right side of the mid-rib and one hun-

dred and thirty-eight eggs on the left side of the mid-rib, leaving a difference of only four eggs. Undoubtedly the female does not prefer either side of the mid-rib in depositing her eggs, it being simply a matter of chance on which side of the leaf she happens to alight.

The position of the receptacle within the leaf is nearly constant; it is usually found above the lower epidermis. Among the one hundred leaves of the peach-leaved willow there were no receptacles cut below the upper epidermis, but among the one hundred leaves of the long-leaved willows there were sixteen leaves with eggs deposited below the upper epidermis and three leaves containing eggs both above the lower and below the upper epidermis. The total number of eggs deposited below the upper epidermis was sixty-three, out of which fifty-six eggs hatched. Undoubtedly the deposition of the egg below the upper epidermis of the leaf does not hinder the development of the eggs.

It is worthy of notice that the saw-fly does not deposit her eggs indiscriminately on all the leaves of the willow. On the various species of willows which we examined no eggs were ever found on the younger leaves of the plant. Perhaps this avoidance of the youngest leaves may be because of their too rapid expansion, which would interfere with the development of the ovum.

The number of eggs one female deposits may exceed five hundred. A dissection of the female reproductive organs shows that each ovary is made up of a variable number of ovarian tubules or ovarioles. In six specimens dissected, the average number of ovarian tubules in the right ovary was twenty-six and in the left twenty-five, although as many as thirty and sometimes only twenty-one were found. The following table shows the number of ovarioles found in each ovary and the total number found in both ovaries of the six specimens dissected.

TABLE II.

Ovarioles in right ovary.	Left ovary.	Total in both.
23.....	23	51
21.....	23	44
27.....	25	52
25.....	27	52
25.....	24	49
30.....	28	58
Average 26	25	51

Alternating with each egg-chamber is a nutritive or yolk-chamber; these can be distinguished as such, with the naked eye, only at the basal end of the ovarian tubule by the larger size of the egg-chambers. An examination of ovarioles mounted *in toto* shows there are about eight to ten well defined egg-chambers alternating with the same number of nutritive chambers. If all of these well defined eggs were to reach maturity, the females would be able to deposit between four and five hundred eggs; but since many more may be developed from the terminal filament, the question of the number of eggs that a female may lay remains an open one.

PARASITE OF THE EGG.

There is a species of Hymenopteron which is parasitic on the eggs of *Cimbex americana*. It is a true internal parasite of the egg, passing all stages, excepting the imago, within the egg. Mr. C. T. Brues has kindly identified this species as *Trichogramma pretiosa* Riley. This Hymenopteron according to Girault (2) attacks no less than eleven or more hosts. A glance at the following list shows that the parasite attacks eggs of insects belonging to families which are often not very closely related. Undoubtedly the Chalcis-fly has a wide distribution.

LIST OF HOSTS ATTACKED IN THE EGG STAGE BY *TRICHOGRAMMA*
PRETIOSA RILEY.*Hymenoptera.*

Pachynematus palliventris Cresson. (record ?)

Pteronus ribesii Scopoli.

Lepidoptera.

Alabama argillacea Hübner.

Autographa brassicae Riley.

Carpocapsa pomonella (Linnaeus).

Heliothis obsoleta Fabricius.

Ianassa lignicolor Walker.

Laphygma frugiperda Smith and Abbot.

Mamestra picta Harris.

Phlegethontias sexta Johanssen.

Platynota rostrana Walker.

Polychrosis viteana Clemens.

The total number of eggs deposited on one hundred leaves of the peach-leaved willow was three hundred and ninety-five, of which only five eggs were parasitized by *Trichogramma pretiosa*, whereas upon the long-leaved willow there were two hundred and eighty eggs deposited, and of these, six were parasitized by this same Hymenopteron. The percentage of eggs which failed to hatch on account of this egg-parasite was therefore less than two per cent.

Besides the eleven parasitized eggs, there were sixty eggs which failed to hatch, out of the six hundred and seventy-five eggs examined. A few observations which we made may explain the destruction of some of these eggs. A number of females were allowed to deposit eggs on a peach-leaved willow and their development was observed from day to day. During the eight days of their development there were heavy rains, and, for some reason or other, a few days before hatching the epidermis of the receptacle of many of the eggs had been broken and curled up, oftentimes exposing the eggs. Heavy rains and strong winds prevailed during the night of the seventh and morning of the eighth day. A visit during the eighth morning

revealed to us that some of the eggs had been washed out of the injured receptacles. A number of leaves with these injured receptacles were taken out of the rain and most of them hatched by five o'clock in the afternoon. The next morning we again visited the trees and found that many of the eggs contained within the injured receptacles had hatched. Another observation might be mentioned which may explain the cause of the destruction of some of the eggs. Occasionally a small red mite was noticed within a broken receptacle busily engaged with the egg, but whether or not these mites injured the eggs the observations thus far made did not determine.

Within five parasitized eggs we found on an average twenty-two pupae of *Trichogramma pretiosa*, although as many as thirty and sometimes only fifteen were found. The number of pupae within each parasitized egg was, respectively, 15, 18, 22, 25 and 30.

The color of an egg, which contains full-grown larvae or pupae, is black (Fig. 1, *p*). This color persists very distinctly, even after the parasites have emerged, and always serves to distinguish a parasitized egg. The parasite escapes by cutting a round, jagged hole through the shell of the host egg and epidermis of the receptacle. These exit holes are rather numerous, the insect in all cases thus far examined cutting its way through the lower epidermis of the receptacle and not through the upper epidermis. We have, as yet, not found a single parasitized egg below the upper epidermis, and it would be interesting to know through which side of the leaf the parasite bores out, if such cases occur. Quaintance and Brues (8) find in the case of the cotton boll worm that, "although several parasites (*Trichogramma pretiosa* Riley) may come from a single egg, generally but one exit hole is present, it being in most cases on one side." When the parasite emerges, it often leaves whitish specks of excrement on that part of the lower epidermis which covers the blackened host egg.

DEVELOPMENT OF THE EGGS.

When the egg is first deposited above the lower epidermis, it is discovered from the upper side of the leaf only with great

difficulty; but when the under surface of the leaf is examined, one can readily observe a slight elevation of the epidermis. Within a few days the elevation is increased, and, as has been previously noted by Comstock (1) and Harris (3), in some strange way, perhaps by the absorption of moisture from the leaf, the egg increases in size until it becomes twice or three times its former size. The receptacle itself becomes enlarged, and a few days before hatching the outline of the future larva is easily seen through the lower epidermis, bent upon itself around one pole of the egg.

On July 14, six specimens were allowed to deposit eggs on a peach-leaved willow and their development was observed from day to day. On July 22, eight days after the deposition, all the eggs with the exception of a few hatched. We had the opportunity of observing the manner in which the larva escapes from its receptacle. Whether the larva first eats its way through the thin membrane which encloses it, or whether this thin shell is broken by the growth or movements of the larva, we are not certain. As soon as the head is free from the egg, one can easily see a pair of brown mandibles cut a semi-circular lid through the thin epidermis of the receptacle (Fig. 2, *a*). The larva, without stopping to eat the membrane which encloses it, pushes up the lid and makes its escape.

THE LARVA.

One would naturally suppose that the larva would escape from the mouth of the receptacle, but if this opening is examined rather closely one is struck with surprise to find that the epidermis around the mouth of the receptacle has grown together with the parenchymatous tissue. The question which now arises is, Why does not the rest of the epidermal layer of the receptacle, not occupied by the egg, grow together with the parenchymatous tissue? We can give only one possible explanation in answer to this question. As already mentioned, after the female has deposited an egg, it injects a certain amount of liquid from the collateral sac into the receptacle. The use of this fluid, perhaps, prevents adhesion between the epidermis and the parenchymatous tissue.

A newly born larva is white, with the middle region of the body yellow, due to the contents of the alimentary canal. The head is pale gray with two prominent black spots, the ocelli. The mandibles are brownish in color. Along the sides of the body are whitish specks; these are the spiracles. The skin is naked, being without hairs, but fine transverse wrinkles are present. When the body is stretched out, as in walking, the larva measures about seven millimeters in length.

A few hours after hatching, even when the larva is not fed, it becomes pigmented. The head turns a dark gray, while the middle portion of the body becomes yellowish-green, which, near the head and anal region, passes gradually over into a white. The spiracles, instead of remaining whitish specks, turn black.

After feeding, the body becomes even darker, assuming a grayish color with a green tinge due undoubtedly to the food material in the alimentary canal. Posterior to the head, the body is a dirty white color, while near the anal region the body is darker, due to the contents of the rectum. One noticeable peculiarity is that the larva becomes covered with a white, flaky substance which rubs off very easily. A newly hatched larva does not, as the more mature larvae have the habit of doing when disturbed, spurt out a watery fluid from certain little pores situated on the sides of the body just above the spiracles.

The larvae were fed daily upon the leaves of the peach-leaved willow. On July 19, five days after the larvae were born, the first moult occurred. The larvae do not eat the skins cast off. An examination of the moulted skin shows that a split extends from the clypeus upward along the median line to the dorsal region of the head, and then backward a short distance through the dorsal median line of the body, the posterior region of the larva being withdrawn from the skin. One peculiarity of a larva which has just moulted, and also of a larva which is newly born, is the head, which in proportion to the rest of the body is exceedingly large. Soon after the larva has moulted the spiracles are hardly discernible, but a few hours later they appear as prominent black specks. After the

moult, the head and body are of a light yellowish color, with a darkened region extending along the dorsal median line of the body. The darkened region is probably due to the contents of the alimentary canal. The larva after moulting measures between eleven and twelve millimeters, when the body is stretched out. A day later, the larvae were again found to be covered with a white, flaky substance. As the life history of *Cimbex americana* is well known, no attempt was made to follow the larvae further than the first moult.

INJURY TO THE WILLOWS.

If one wishes to know whether specimens of *Cimbex americana* are found in a certain locality, all that is necessary is to examine the branches of various species of willows, and if rough gashes extend transversely or nearly so around the limbs, one may be certain that this insect is the cause of the injury. These gashes, which the saw-flies cut with their strong mandibles, extend spirally around the terminal twigs (Fig. 3, *sc*), but around the somewhat thicker branches the incisions often extend completely around the limb (Fig. 3, *tc*), or more often a little more than halfway around the twig, or there may be a number of smaller incisions one above the other. The incisions generally extend through the bark and may be cut a slight distance through the wood.

An examination of some of the branches one to three or four years after the injury shows that the incisions have always healed over, although sometimes a splitting of the bark may have occurred at the place of injury (Fig. 4, *b*). The tree continues to grow, apparently without being injuriously affected. In only a few cases did we notice branches broken at the incisions, although if one bends a branch which *Cimbex* has attacked, it always breaks at the incision, and often with little exertion.

What can be the object of cutting these incisions in the twigs? Comstock (1) writes as follows:—"These saw-flies have been known to injure willows by biting incisions half-way around the terminal twigs of the tree. What their object was

in doing this is a mystery." In a letter to F. M. Webster, published in *Insect Life* (10), R. M. Pritchard writes:—"I found a small number settled on the leaves and limbs of the ash and willows, where they seem to be feeding on the sap. Today I have been watching them more carefully and find that they cut a rough gash almost completely around the limb, seeming to kill the outer bark as far as they cut." Webster, at the end of the paper, adds:—"It seems quite possible that they gnaw the bark for the purpose of feeding upon the sap, as intimated by Mr. Pritchard, yet this does not appear to be fully proven. In other words, it would as yet be too much to say that in cutting the incisions the insect has no object in view other than that of obtaining food." While observing a number of saw-flies in the act of cutting incisions, we chanced to come across a twig from which sap was oozing from a freshly cut wound. About half a foot below the wound a female saw-fly was started up the twig. While she was walking slowly up the branch, we noticed that the palps were continually in motion. When the insect came to the sap, she suddenly stopped and began to feed upon the liquid. While working on the anatomy and histology of the alimentary canal, we often noticed bits of bark in the lumen of the crop. Some of the specimens which were dissected had the crop filled with a clear liquid. Undoubtedly the only object which the saw-fly has in view in making the incisions is to tap the fibro-vascular bundles and thus secure its nourishment.

If one examines the leaves shortly after the eggs have hatched, one often notices rusty spots on the upper and lower sides of the leaf. Frequently, however, these rusty spots are entirely absent in those leaves wherein eggs had been deposited. Then, again, one may find, especially on those where the egg for some reason or other failed to hatch, a dense fungous growth within the receptacle.

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EXPLANATION OF PLATE V.

All figures, except Figure 5, are drawn natural size.

Fig. 1. Ventral surface of leaf of peach-leaved willow, showing an egg in each completed receptacle:—*r*, receptacle; *3r*, three receptacles which communicate with one another; *mr*, mouth of receptacle; *pr*, partially completed receptacle; *e*, egg; *p*, egg parasitized by *Trichogramma pretiosa*; *i*, incision.

Fig. 2. Ventral surface of leaf of long-leaved willow, showing the semi-circular lid which the larva pushes up in making its escape:—*a*, lid of receptacle; *r*, receptacle; *mr*, mouth of receptacle; *pr*, partially completed receptacle; *i*, incision.

Fig. 3. Gashes or incisions which the saw-fly cuts in the twigs:—*sc*, spiral incision; *tc*, incision which extends completely around the branch; *c*, gash which extends only partly around twig.

Fig. 4. A willow branch, a number of years after the injury, showing the incision healed over but with a splitting of the bark at *b*.

Fig. 5. *Trichogramma pretiosa* Riley, parasite of the eggs of *Cimex americana* Leach.

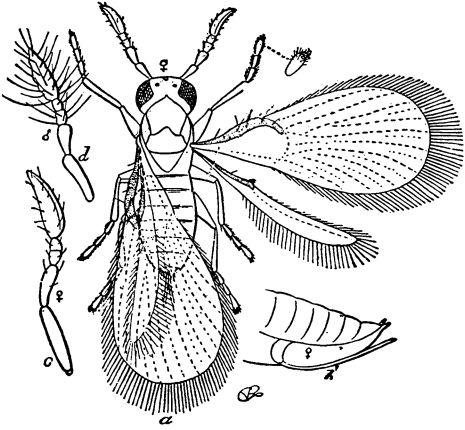
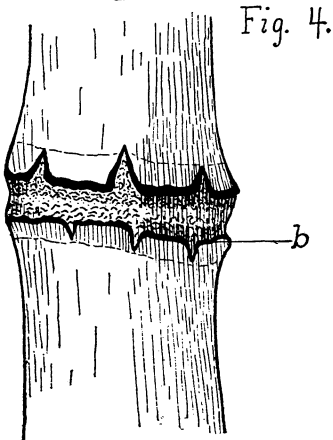
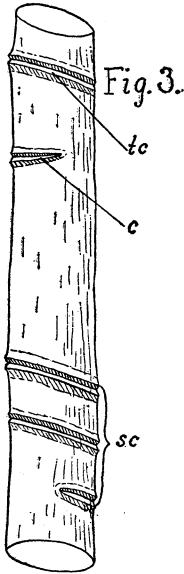
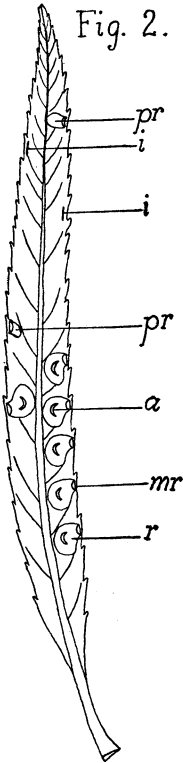
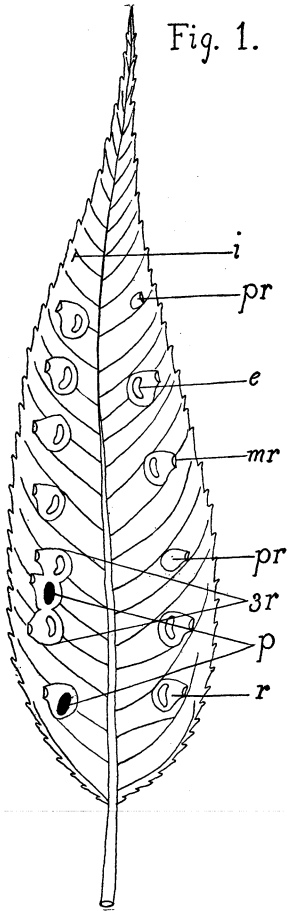
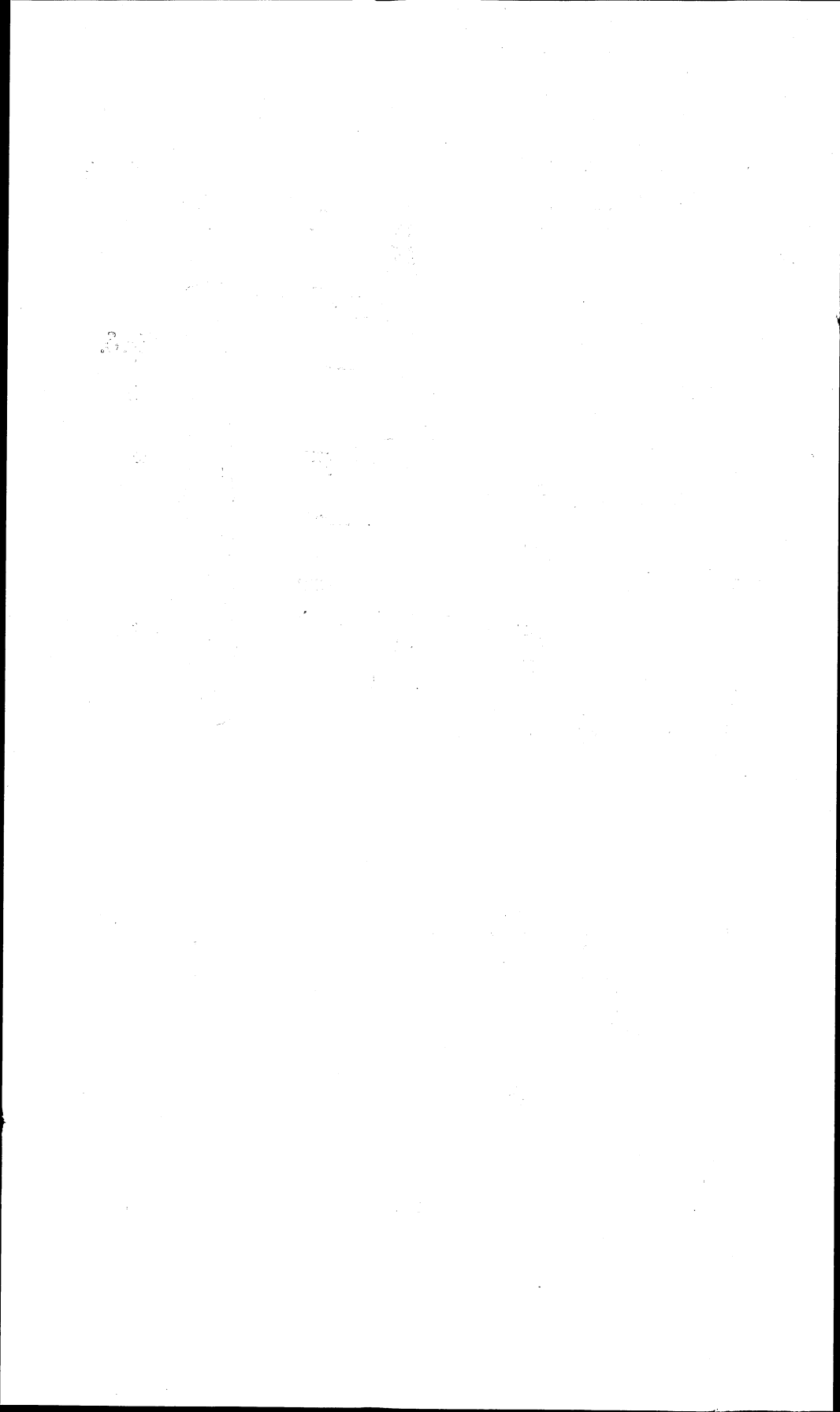
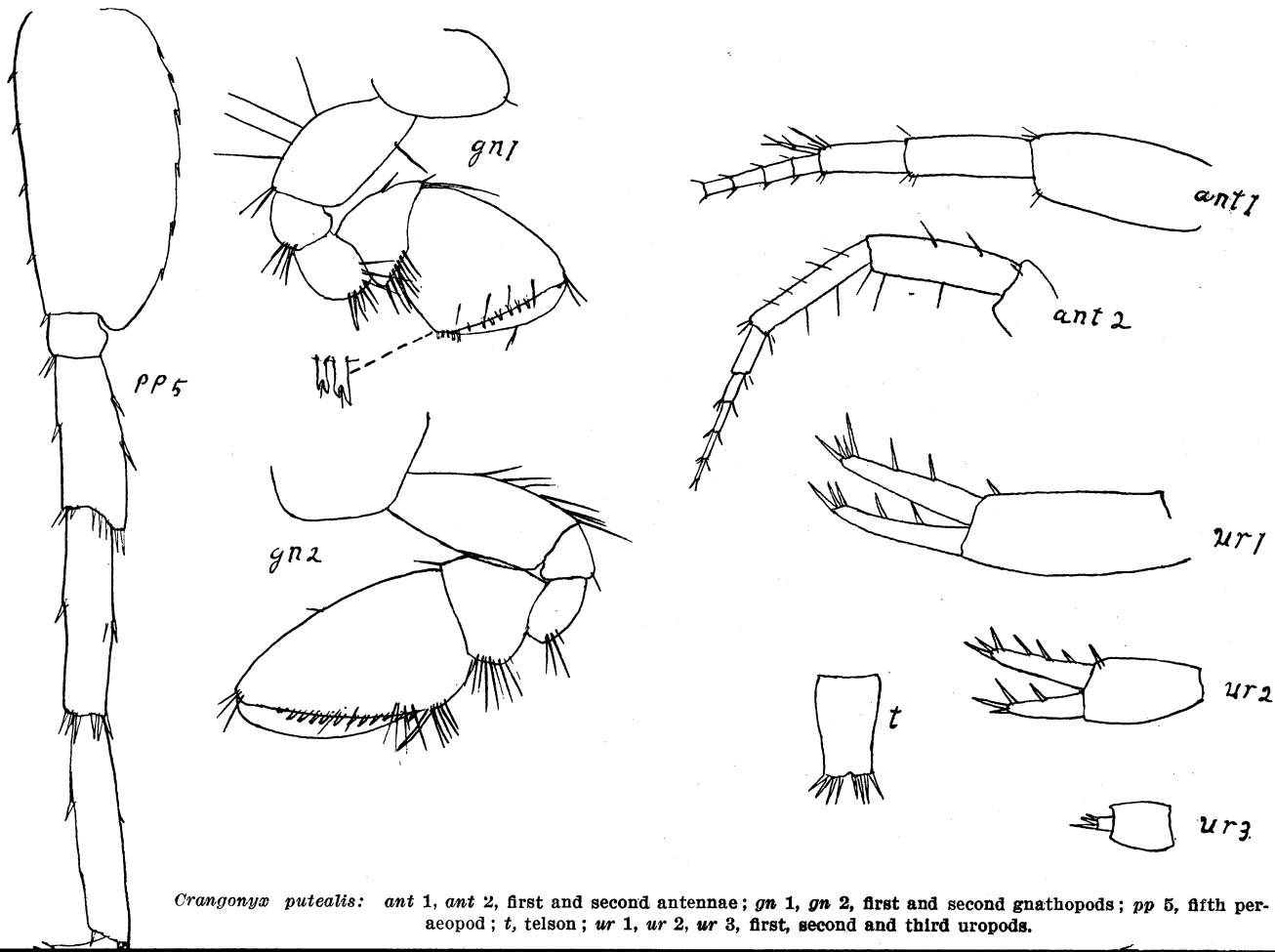


FIG. 5. — *Trichogramma pretiosa* (from Riley).



[illegible]



Crangonyx putealis: ant 1, ant 2, first and second antennae; gn 1, gn 2, first and second gnathopods; pp 5, fifth pereopod; t, telson; ur 1, ur 2, ur 3, first, second and third uropods.

DESCRIPTION OF A NEW SUBTERRANEAN AMPHIPOD FROM WISCONSIN.

S. J. HOLMES.

(With Plates VI and VII.)

Crangonyx putealis sp. nov. (Plate VI).

Body slender; side plates low. Eyes absent. First antennae about half the length of the body, the second joint of the peduncle a little shorter than the first, the third about two-thirds the second; flagellum longer than the peduncle; secondary flagellum consisting of two joints. Second antennae a little over half the length of the first, the last two joints of the peduncle subequal; flagellum but little longer than the last joint of the peduncle and consisting of five or six joints.

First gnathopods stout; hand nearly as broad as long, distally widened, the palm oblique and armed with several short spines, each of which bears a single seta in a terminal notch; posterior margin devoid of setae. Second gnathopods longer and relatively more slender than the first; hand narrowly ovate with very oblique palm armed with notched spines, a long spine at upper end of palm; posterior margin, except near the upper end of the palm, devoid of setae.

First pair of uropods extending beyond the others, the rami subequal, a little shorter than the peduncle, and armed with two spines on the upper margin and a cluster of three or four spines at the tip. Peduncle of the second uropods with a spine at the distal end of the upper margin; inner ramus about as long as the peduncle, armed with about three spines on the upper margin and three or four at the tip; outer ramus a little

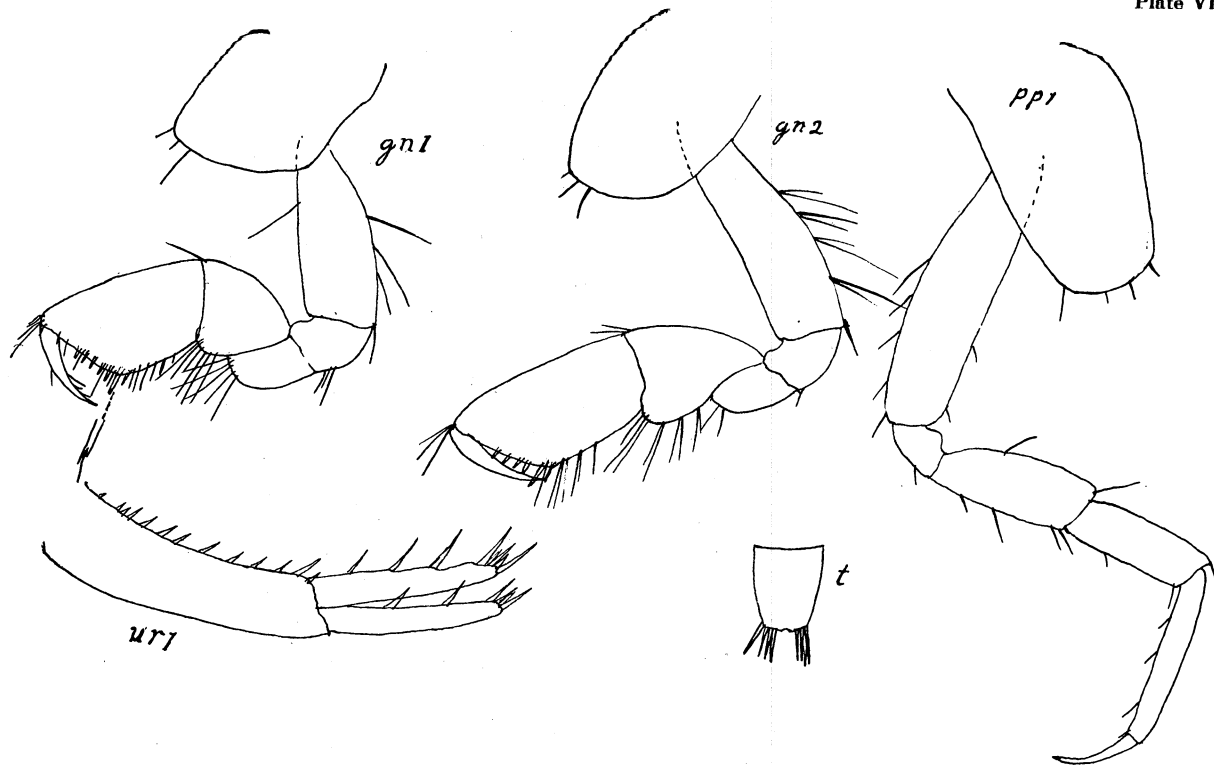
shorter than the inner, armed with three terminal spines and a single spine on the upper margin. Third uropods with a short, thick peduncle devoid of spines; the single small ramus less than half the length of the peduncle and armed with three spines at the tip.

Telson about twice as long as wide, subrectangular but slightly narrowed distally, the posterior margin with a slight emargination on either side of which is a cluster of four or five spines.

Length, 5 millimeters. All the specimens were entirely devoid of pigment. The gnathopods do not present any marked differences in the two sexes.

This species differs from *Crangonyx tenuis* Smith from Connecticut in having considerably longer rami in the first uropods, in having the outer ramus of the second uropods but little shorter than the inner, instead of half as long, and in having a much shorter flagellum in the second antennae. It is more closely allied to *C. vitreus* (Cope) from Mammoth Cave, Kentucky, so much so that I was at first somewhat in doubt of its specific distinctness. Through the kindness of Mr. Richard Rathbun, who sent me some specimens of the latter from the United States National Museum, I was enabled to compare the two species and to assure myself that they are really different. In *C. putealis*, the first gnathopods are considerably stouter than in *vitreus*, the hand is more widened distally and has no setae on the posterior margin; the hand of the second gnathopods has the palm more oblique, and a shorter posterior margin which is devoid of setae except near the palm; the first uropods have fewer spines on the upper margin of the peduncle, and the telson is relatively longer, being nearly twice as long as wide, whereas in *C. vitreus* its width nearly equals its length. Camera drawings of parts of *C. vitreus* are inserted for the sake of comparison (see Plate VII).

It is a noteworthy fact that, of the seventeen species of fresh-water Amphipoda known to occur in North America, ten are inhabitants of underground waters. Some of these occur in caves, such as *Gammarus purpurascens* and *Niphargus an-*



Crangonyx vitreus, female from Mammoth Cave; *gn 1*, *gn 2*, first and second gnathopods; *pp 1*, first pereopod; *t*, telson; *ur 1*, first uropod.

[illegible]

10. *Chlorophyll a* (Chl *a*)

tennatus from Nickajack Cave, Tennessee, *Crangonyx vitreus* from Mammoth Cave, Kentucky, and *Gammarus caecus* from a cave in Cuba. Besides the species here described, the following have been found in wells: *Apocrangonyx lucifugus* Hay from Knox County, Illinois, *Stygonectes flagellatus* (Benedict) from San Marcos, Texas, *Crangonyx tenuis* Smith from Connecticut, *Eucrangonyx packardii* (Smith) from wells in Orleans and New Albany, Indiana, and *Eucrangonyx mucronatus* (Forbes) from wells in Indiana and Illinois. *Gammarus propinquus* Hay, which has been taken from springs near Mammoth Cave, Kentucky, in Missouri, and in West Virginia, is probably also to a considerable extent a subterranean form. None of the species mentioned as coming from wells has thus far, I believe, been reported from any other habitat.

It will be seen that the underground species of Amphipoda are quite widely distributed. Many of the species are rather closely related to forms inhabiting the surface waters of neighboring regions. *Gammarus purpurascens*, for instance, strongly resembles the widely distributed *G. limnæus*, and *Eucrangonyx packardii* is a close ally of *E. gracilis*, which occurs in most of the northern states east of the Rocky Mountains, differing from it chiefly in the absence of eyes. *Apocrangonyx lucifugus*, *Stygonectes flagellatus*, and the three species of *Crangonyx* found in North America, although placed in different genera, are not widely different from the species of *Eucrangonyx* living in lakes and streams. *Niphargus antennatus* has no very close relatives among the surface forms, although it belongs to the same family, Gammaridae, which includes the other species here mentioned; but there are several species of *Niphargus*, most of which are underground forms, which occur in Europe.

The fresh-water Amphipoda possess a remarkably strong proclivity toward an underground life. They are notoriously creatures of retiring habits, seeking shaded and protected nooks out of reach of fish and other enemies that prey upon them. They are, as a rule, negatively phototactic, and they possess a strong positive thigmotaxis—two traits that conspire to lead them into a subterranean habitat when opportunity offers.

The existence of these various subterranean species in so many remote localities suggests that the underground fauna of North America may be more extensive than is commonly suspected, and that there are numerous underground highways of dispersal of considerable extent. So far as I can ascertain, none of the subterranean species of North America has been taken in the surface waters except *Gammarus purpurascens*, which was originally described from Nickajack Cave, Tennessee, but was also found to occur in a spring at Rossville, Georgia. In the latter locality it was not improbably brought up from below. With the exception of *Gammarus purpurascens*, all of the subterranean Amphipoda of this continent are either blind or have the eyes in a rudimentary condition,—a fact which indicates that they are permanently subterranean forms and not accidental inhabitants of underground waters.

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CONCERNING THE ORGANIZATION OF THE SPORE MOTHER-CELLS OF *MARSILIA QUADRIFOLIA*.

W. MARQUETTE.

(With Plates VIII and IX.)

In a recent paper,¹ I incidentally called attention to the fact that during the synapsis stages of spore mother-cells of *Marsilia quadrifolia* the starch grains, which are almost invariably present at that time, are aggregated in a definite region of the cell. In connection with the translocations which the starch grains in young leaf-cells of *Isoetes* undergo during mitosis, this raised the question as to the behavior of the starch aggregation in the spore mother-cells of *Marsilia* during mitosis, and as to whether there is a permanent polar organization of the cells here similar to that found in the vegetative cells of *Isoetes*. Further interest is given to a study of the organization of the cells in the sporocarp of *Marsilia* because of Belajeff's statements² regarding the behavior of the blepharoplasts in the microgametophytes of *Marsilia*. Belajeff traces the blepharoplast back several cell-generations from the antherozoid and describes it as behaving during mitosis in all respects like the central body of animal cells, and concludes that they are similar in function.

It is to be expected that a study of the method of spindle formation and cell division in the various cells of the sporo-

¹ Manifestations of polarity in plant cells which apparently are without centrosomes. *Beih. z. Bot. Centralbl.*, xxi, Abt. i:297. 1907.

² Belajeff, W.: Ueber die Centrosome in den spermatogenen Zellen. *Ber. d. deut. bot. Ges.*, xvii:199. 1899.

carp will throw light on the question as to the presence in the sporocarp cells of the blepharoplast, or of structures related to it and finally giving rise to it. It was with these problems especially in view that I undertook the investigations here described.

The material was obtained from plants of *M. quadrifolia* growing in the greenhouses connected with the botanical laboratories of the University. Flemming's stronger and Flemming's weaker chrom-osmium-acetic fixing fluids, as well as some modifications of the same, were used for the most part and gave the best results. In general, the original formulae as published by Flemming gave at least as good if not better results than the various modifications of the same which have from time to time been recommended. Several sublimate mixtures were also used but did not give results equal to those of the chrom-osmium-acetic mixtures, although they gave valuable control preparations. For staining, Flemming's triple stain and Heidenhain's iron-haematoxylin were used almost exclusively. The sporocarps of *Marsilia* offer a most favorable material for cytological study. They are readily obtained in all stages of development, fix well, and, contrary to what might be expected, section easily. The different parts of the cell take a good differential stain with the triple stain, so that preparations of unusual brilliancy result.

Furthermore, the cells found in the sporocarp offer an interesting variety. There are the ordinary vegetative or somatic cells of the sporocarp walls, etc., which may be looked upon as typical vegetative cells. In young sporocarps these are actively dividing, so that abundant instances of the various stages of spindle formation are to be found. The tapetal cells represent another type of somatic cells, i. e. cells which are soon to disintegrate. For such cells, aberrant nuclear and cell divisions have been described for a large number of plants and animals. Then there are the "germ cells," the spore mother-cells and their ancestors. In the spore mother-cells we have cells which have severed their structural connection with other cells, so that they do not form an architectural part of a tissue, at least not in the later stages of their development when they are begin-

ning to divide. A tissue cell is subject to specific and different environmental conditions on its different sides, certain factors of which remain constant for at least considerable periods. With the free spore mother-cells it is otherwise. While the conditions are not the same in all directions in the spore sac, any part of a free spore mother-cell apparently is as likely to lie in a given position as any other; furthermore, there is some evidence that the spore mother-cell rotates more or less previous to and during division, so that, whatever differences there may be in the environment of a spore mother-cell, there are no fixed and specific differences with respect to the environment between different parts of the cell, as is the case with cells forming part of a tissue complex, and in this difference a possibility is provided for a difference in the method of formation and orientation of the karyokinetic figure in the two cases.

In the following, I shall take up only the organization of the spore mother-cells and the processes of division occurring in them. I have concerned myself especially with the activities of the cytoplasm. The points in Strasburger's paper,³ which appeared when my work was practically completed, bearing on the problems taken up here will be considered as they come up.

Shortly after their formation, the sixteen spore mother-cells of a spore sac each show an approximately centrally placed nucleus in a dense cytoplasm. The more solid constituents of the cytoplasm are closely packed and usually show no inclusions at this time. Nucleus and cell are of relatively small size as compared with later stages. A period of growth now sets in during which the size of the nucleus and cell increase greatly up to the time of synapsis. During this period of growth, specific and characteristic changes occur in the cytoplasm. At what is apparently a variable period in the growth of the spore mother-cell, starch grains make their appearance in the cell. Just before they appear, the cytoplasmic strands, or lamellae, of the spore mother-cell are relatively coarse and of irregular outline. For the most part, these

³-Strasburger, E.: Apogamie bei *Marsilia*. *Flora*, xcvi:123. 1907.

strands tend to take the orange of the triple stain, but here and there are portions staining blue. In some cases, larger, blue-staining granules are found at intervals along the strands. On the outer side of the cell, in the position finally to be occupied by the starch mass, a relatively large, elongated, clear region is frequently to be found. At times it is of regular outline, lying about midway between nucleus and outer cell wall, its short diameter equal to about one-quarter the distance between the nucleus and cell wall. At other times this region is more irregular in outline and may branch somewhat. In any case, it is bounded by comparatively heavy, blue-staining elements of the cytoplasm which frequently show larger granules lying close against their inner side; the upper left corner of Figure 2 shows part of such a region. The appearance of this region in the cytoplasm suggests the polar structures of *Isoetes*, especially as they are found in the early pro-phases and practically free from starch. Unfortunately, I am at present unable to say anything further concerning the significance or subsequent history of this appearance in the cytoplasm of the spore mother-cells of *Marsilia*. The nucleus at this time shows the chromatin finely divided and aggregated in clusters of varying shapes; frequently long, irregular threads of chromatin run out from these clusters. Numerous nucleoles are present.

When the starch grains first appear, they are usually more or less scattered in the cell and rather widely separated from each other; frequently it seems they tend to lie near the nucleus. Soon, however, this scattered arrangement gives place to a more definite one; the starch grains are found at a specific region of the cell, being located almost invariably between the nucleus and the wall of the cell facing towards the outside of the spore sac. Frequently the starch grains lie in a quite distinctly bounded group, often of a cylindrical shape, one end in contact with the nucleus and the other reaching almost to the cell wall (Fig. 3). While most of the starch grains at this stage lie in this region, it is to be noted that usually some of them lie outside of it. In the cell represented in Figure 3, in a higher plane than that at which the

drawing is made, a number of starch grains, arranged in a single row, extend fully halfway around the nucleus.

The starch grains at this time stain a deep blue, and are not as closely packed as at a somewhat later stage. The more or less cylindrical region lying between nucleus and outer cell wall contains, in addition to the starch grains, strands with a granular structure which run here and there between the starch grains, probably forming more or less of a network. Besides these strands, there seems to be a diffuse, finely granular, blue-staining substance scattered throughout this region. It is difficult to make out with certainty the structure of the material between the starch grains, because the latter are numerous and stain heavily, so that at any particular focus considerable light is cut off by the overlying and underlying portions; but it seems certain that there is a finely divided substance staining blue in the triple stain between the starch grains. The abrupt ending of this material outside of the starch grains seems to be at least to a considerable extent responsible for the fairly sharp delineation of this region against the remainder of the cytoplasm. The cytoplasm as a whole is still rich in its more solid constituents, although the proportion of vacuolar space has become somewhat larger than it was in the earlier stages. The strands have become more drawn out, look more like fibers, and many of them show perhaps a greater affinity for the blue stain. Also conspicuous at this time are unusually long strands, which frequently lie in groups, converging at one end and spreading apart at the other. In addition, there are dense-staining granules scattered here and there in the cytoplasm.

The cell apparently has reached its full size, although the nucleus, which at this stage lies near the inner cell-wall, seems as yet noticeably smaller than at the time of synapsis. The chromatin is in the form of strands of fairly regular outline and uniform diameter, which loop and coil around considerably, often traversing the nuclear cavity. Free ends of such strands are not apparent, although if they lay at the points where the strands come in contact with the nuclear membrane they could easily be overlooked.

The starch masses of the sixteen spore mother-cells in a spore sac are placed so as to be removed as far as possible from each other. This suggests that the position of the starch mass in the cell may be determined by metabolic requirements. The region of the starch mass is probably at times the seat of great metabolic activities in the cell. At least the greater part of interchange of materials between the spore mother-cells and the exterior occurs through the tapetum; on the other hand, there is considerable evidence that the nucleus is intimately concerned with the metabolic activities of the cell.⁴ Now, the location of the starch masses of the sixteen spore mother-cells of a spore sac is such that they lie in a position of maximum advantage with reference to an interchange of metabolic materials and products with the surrounding tapetum on the one hand and to proximity to the nucleus on the other.

It is to be noted that the spore mother-cell at this stage presents a distinct polar organization. A line drawn through the center of the starch aggregation and through the center of the nucleus would represent the main axis of the cell. The first conspicuous evidence of this organization is the appearance of the starch mass at a definite place in the cytoplasm. It is to be noted further that the nucleus itself as yet shows no evidence of polarity. In synapsis, however, which soon sets in, the nucleus presents several most interesting features bearing on its organization at this time. The synaptic mass, as is commonly the case, collects on one side of the nucleus, and, as I previously noted, in *Marsilia* it is invariably on the side of the nucleus next to the starch aggregation in the cytoplasm. This stage is of long duration, so that great numbers of cells are found in this condi-

⁴ Küster (Ueber die Beziehungen der Lage des Zellkerns zu Zellenwachstum und Membranbildung. *Flora*, xcvi:1, 1907) has recently thrown doubt on Haberlandt's work (Beziehungen zwischen Function und Lage des Zellkerns bei den Pflanzen, Jena, 1887) concerning the relation between the nucleus and the metabolic activities of the cell. There are, however, numerous other data pointing to such a relation. I have in mind especially the results obtained on secreting cells, particularly animal cells.

tion.⁵ Usually the chromatin strands can be quite readily followed throughout this stage, but at times unusually dense synaptic masses are met with in which the individual chromatin strands are practically indistinguishable. A striking feature of synapsis in *Marsilia* is that a large number of the strands converge toward a point on the nuclear membrane which lies on, or close to, the axis of the cell. Furthermore, at this point, closely in contact with the nuclear membrane, lies a nucleole, sometimes apparently two, differing from the other nucleoles of the nucleus. This comes out especially well in preparations stained with the triple stain. Usually a considerable number of the nucleoles are still present at this stage, and the one lying at the focal point of the chromatin strands almost invariably shows a bright, deep red color, standing out sharply from the blue chromatin strands. The other nucleoles lying here and there in the nuclear cavity, on the other hand, show a somewhat pale grayish-blue color. These figures are frequently of remarkable regularity, standing out with almost diagrammatic clearness.

The chromatin strands during synapsis frequently show more or less of a characteristic orientation. In animal cells, the strands are centered upon the central body and the synaptic mass lies against the nuclear membrane on the side next to the central body.⁶ The same is true of the Ascomycete *Phyllactinia*.⁷ In the higher plants, the synaptic mass usually lies close to one side of the nuclear membrane, but in general the

⁵ As this was ready to go to press, my attention was called to a paper by Schaffner (Synapsis and synizesis. *Ohio Naturalist*, vii:41, 1907), in which he describes and figures synapsis in a sporocarp of *M. quadrifolia* and points out that the synaptic mass lies on the side of the nucleus nearest the sporocarp wall, attributing this orientation, however, to the action of the fixing reagents. There is no occasion to enter into a discussion of this and other similar conclusions of the author concerning synapsis in general.

⁶ Farmer, J. B., and Moore, J. E. S.: The meiotic phase in animals and plants. *Quart. Jour. Microscop. Sci.*, xlviii, Pl. XL, Fig. 72. Schreiner, A. und K. E.: Ueber die Entwicklung der männlichen Geschlechtszellen von *Myxine glutinosa*. *Arch. de Biol.*, xxi, Figs. 52, 71, 170, etc.

⁷ Harper, R. A.: Sexual reproduction and the organization of the nucleus in certain mildews. *Carnegie Inst. Publ.* No. 37 (1905), Pl. IV, Figs. 43-45.

figures which have so far been given of synaptic stages show no conspicuous centering of the chromatin strands upon any particular region, although occasionally a nucleus has been figured showing a rather conspicuous orientation of the strands upon a certain part of the nuclear membrane; for example, by Overton for *Helleborus foetidus*⁸ (in this case the nucleole is included in the synaptic mass but does not lie in contact with the nuclear membrane), and by Berghs for *Narthecium ossifragum*.⁹ In the latter case the strands are centered upon a nucleole lying at the periphery of the nucleus. But in other figures of the same plants given by these authors the nucleoles lie outside of the synaptic mass and the chromatin strands show a less regular course. In *Lilium*, which has been so extensively studied,¹⁰ there seems to be little indication of an orientation of the strands upon any one region, nor is there any regularity in the position of the nucleole. In *Marsilia quadrifolia* the orientation of the chromatin strands during synapsis is apparently as definite and characteristic as in *Phyllactinia* or in any of the animal cells.

The nuclear membrane also shows an interesting dissimilarity between its "polar" and "antipolar" sides. This difference stands out especially well in preparations which are stained rather faintly. The nuclear membrane appears much heavier on the side of the nucleus turned away from the starch mass than on the side next to it. In some cases it almost looks as if a close-fitting cap extending over one-third to two-thirds the surface of the nucleus were symmetrically placed opposite the synaptic mass over the otherwise thin nuclear membrane. If the preparation has a slight excess of the orange stain, this part continues to stain blue and stands out in contrast to the otherwise largely yellow preparation. Whether there actually is a thickening of the nuclear membrane here is perhaps a question. In a normally stained preparation it is seen

⁸ Overton, J. B.: Histologische Beiträge zur Vererbungsfrage. *Jahrb. f. wiss. Bot.*, xlii (1906), Pl. VII, Fig. 46.

⁹ Berghs, J.: La formation des chromosomes heterotypiques dans la sporogénèse végétale. *La Cellule*, xxii, Fig. 17.

¹⁰ See the figures of Allen, C. E.: *Jahrb. f. wiss. Bot.*, xlii (1906), Pl. II, and *Ann. Bot.*, xix, Pl. VI.

that numerous deeply-stained blue granules lie close against the inner side of this part of the nuclear membrane (Fig. 4). They are rarely if ever more than one layer deep, and, although small, stain intensely, so that they are readily made out in a tangential section of the nucleus. These granules are found only under the apparently thicker parts of the nuclear membrane, and undoubtedly are partly responsible for the heavier appearance of this part. As will presently be seen, the arrangement of the cytoplasmic fibers outside of the nucleus is also such as to make this part of the nuclear membrane appear thicker. Blue-staining fibers have become conspicuous in the cytoplasm and form an intertwining network of relatively long fibers, lying for the greater part close to the nucleus. The fibers have a distinctly different appearance now from that presented by the fibrous constituents of the cytoplasm in the earlier stages of the development of the spore mother-cells. They have become smoother, take a brighter blue stain, and stand out in greater contrast to the other constituents of the cytoplasm; here and there they show thickenings and granules lying against them. In a tangential section taking in just a small piece of the surface of the nucleus, these fibers can be seen in great numbers forming a loose tangle about the nuclear membrane. A median section of the cell, however, taken in such a plane that it includes the nucleole at the focus of the chromatin strands, in other words in a plane including the main axis of the cell, shows that the cytoplasmic fibers are not uniformly distributed about the nucleus (Fig. 4). At the side opposite the starch mass they lie closely packed against the nuclear membrane, so closely that frequently it is difficult to make out the individual fibers; the appearance at times suggests that the cytoplasmic fibers are separating, "splitting off" from this denser part of the nuclear membrane. As they approach the region of the starch mass, the fibers separate more from each other and extend farther from the nucleus. When they reach a plane drawn a little above the nucleole at the focal point of the chromatin strands and perpendicular to the main axis of the cell, the fibers frequently bend rather sharply in towards the edges of the starch mass, at times giving rise to a grouping

of the fibers which suggests the beginning of the formation of spindle poles. It is to be noted that if the completed spindle should lie in this plane, its axis would be at right angles to the original "main axis" of the cell. When the fibers reach the starch mass it is practically impossible to trace them further. Except for the blue-staining fibers, the cytoplasm shows a somewhat looser structure than in the preceding stages.

The close packing of the fibers against the nuclear membrane on the side opposite the starch mass also contributes to make this part of the nuclear membrane appear thicker. However, a close study makes it seem probable that the appearance is due to something more than the mere deposition of granular material on the inside of this part of the membrane and the close apposition of cytoplasmic fibers on its outside; this is indicated, among other things, by the frequently abrupt ending of this region. At any rate, the accumulation of this dense, blue-staining material at a specific part of the nuclear membrane requires further study. The remainder of the membrane is exceedingly delicate, often it is scarcely to be made out. It is to be noted that all this development of the conspicuous fibrous system just described has occurred before and at the time of synapsis.

I have called this stage *synapsis*, although there are certain stages passed through by the nucleus at a much earlier period than this which further study may prove to be of importance in connection with the processes usually associated with synapsis. Early in the growth of the spore mother-cell, when the nucleus is still small, a stage is passed through in which the chromatin is aggregated in a dense mass lying at the center of the nucleus (Fig. 1). The chromatin is at this time in the form of finely divided particles and tends to collect into small, denser groups within the larger mass; often strands of varying lengths are formed, and usually one or several loops of chromatin extend out from the central mass into the surrounding clear nuclear cavity. The whole appearance strongly resembles synapsis. It differs from synapsis as usually found in that in this case the contracted chromatin mass lies at the center of the nucleus instead of at the periphery, and in that it occurs at

a much earlier stage in the growth of the cell than is commonly the case.¹¹ Speaking in terms of current theories, the possibility suggests itself that at this stage the pairing of the homologous chromatin elements may take place, while the subsequent contraction and specific orientation of the chromatin strands is associated with the mechanics of spindle-formation, i. e., with the relation between spindle-fibers and chromatin; and that in this latter conception may lie the explanation of the specific orientation of synaptic masses in general. This specific orientation is as conspicuous as the contraction of the chromatin at this period and is quite unaccounted for on the basis of the theories which find in synapsis a provision for the pairing or fusing of homologous chromatin elements. However, further knowledge of the behavior of the chromatin at this time is needed as a basis for such suggestions. The possibility is not excluded that the early contraction in the spore mother-cells of *Marsilia* may be artifact, although it is too regular in its occurrence to make such a supposition probable. For the present, I shall continue to speak of the later aggregation as synapsis; but it is to be noted that this is preceded by a "spirem" stage!

As the chromatin passes out of synapsis and becomes more uniformly distributed through the nuclear cavity, the fibrous constituents of the cytoplasm become even more conspicuous and usually show a tendency to be arranged in cone-shaped groups of which there are frequently two in a cell. At times a well formed bipolar spindle is developed, the spindle usually lying somewhat tangential to the nucleus (Figs. 5 and 6). Usually this "spindle" lies on the side of the nucleus adjacent to the starch mass. Strasburger¹² figures a similar stage for *M. elata*, and there the same relations seem to obtain between the position of the nucleus, the spindle, and a dense region of

¹¹ Generally in the higher plants the nucleus has reached about half or more of its definitive size (linear) by the time of synapsis, although there are exceptions, as in *Galtonia* according to Miyake [*Jahrb. f. wiss. Bot.*, xlii (1906), Pl. III, Figs. 6-12]. In the case of the primary oocyte in animal cells, there is of course usually a tremendous increase in the size of the nucleus after synapsis.

¹² Apogamie bei *Marsilia*. *Flora*, xcvi, Pl. VI, Figs. 71 and 72.

the cytoplasm. Strasburger does not specifically describe this as a starch-containing region, but judging from the similarity between the appearance of this region and that of what is undoubtedly the starch-bearing region of *M. quadrifolia*¹³ described by him as "eine einseitige Verdichtung im Zytoplasma", it is probable that it is the same in *M. elata*. Occasionally abundant polar radiations extend from the poles to the cytoplasm on the side of the spindle away from the nucleus, and there may be a pronounced crossing of these radiations in the equatorial region. This crossing necessarily occurs near the surface of the starch mass (the fibers apparently in general do not extend into the region occupied by the starch grains), and is especially conspicuous when the section is such that the greater part of the starch mass is cut away, or when the starch is but faintly stained. In the cell represented in Figure 5, the greater part of the starch lies beneath the plane of the drawing. The poles of the spindle, or, perhaps more accurately, the apices of the cones of fibers, usually do not lie in contact with the plasma membrane, but a short distance inside of it. Occasionally dense aggregations appear in the cytoplasm about the poles, at times also a deeply staining granule is found there; however, there seems to be nothing constant in these formations, which apparently occur just as frequently elsewhere in the cell as at the apices of the fibrous cones. The left hand cell in Figure 6 shows at the upper side such a granule which closely simulates a central body with a hyaline zone and a dense aggregation of cytoplasm at its periphery, such as is common in animal cells. In the preparation, this is, if anything, even more striking than in the drawing. As the figure shows, however, this formation in this case seems to be quite independent of the two groups of spindle fibers which have developed, a fact which alone takes it out of the category of the central bodies as they are known in the lower plant and animal cells—unless, indeed, these groups of fibers should not be the beginning of the spindle figure, a possibility for which, as will be seen later, there is some evidence.

¹³ Ibid., Figs. 60 and 61.

By going over a large number of preparations of spore mother-cells, it is possible to pick out a series in which a granule can be shown at all of the various positions which a central body is expected to occupy during the processes of nuclear and cell division, from the stage of the single granule lying close to the membrane of the resting nucleus, and that of its division into two, to the appearance of the single granule lying at the polar side of the reconstructed daughter nucleus. But for every preparation of any stage which shows the formations in question in the expected position, there are dozens in which it either does not appear at all or in which similar formations occur elsewhere in the cell and in varying numbers. There is no occasion to dogmatize concerning these granules and aggregations; it is possible that more complete data will show that they run through a regular cycle and that they play a part in the mechanics of the cell; but as far as our data go at present, there is little or nothing to make this probable. The formation of two cone-shaped groups of fibers at this stage is not invariable. Sometimes a larger number is formed; Figure 5 shows indications of a third cone, and sometimes such sharply defined poles as are shown in Figures 5 and 6 do not seem to be formed at all. While it is common to find the fibers of the cytoplasm collected into two cone-shaped groups at this time, it would appear that they do not directly form the spindle figure. These large cone-shaped groups of fibers are most frequently met with at the time when the chromatin strands are long and thin and show no marked signs of segmentation. Somewhat later, when the thread has thickened considerably and the individual chromosomes are easily made out, the fibers are often in numerous smaller cone-shaped groups, each consisting of but a few fibers. These groups are rarely if ever placed symmetrically about the nucleus, as is the case in the radial stage¹⁴ described for the spore mother-cells of various plants. Instead, the fibers lie more or less tangential to the nucleus on one side, at times suggesting a "spindle wall" as

¹⁴ Osterhout, W. J. V.: Ueber Entstehung der karyokinetischen Spindel bei *Equisetum*. *Jahrb. f. wiss. Bot.*, xxx:159. 1897. Allen, C. E.: The early stages of spindle formation in the pollen mother-cells of *Larix*. *Ann. Bot.*, xvii: 281. 1903.

described by Osterhout for *Agave*,¹⁵ while on the other side of the nucleus the fibers are in small, radially placed, cone-shaped groups whose bases are directed toward the nuclear membrane (Fig. 7). The cell from which Figure 7 is taken shows a number of these groups in a lower plane of the section. As in the preceding stages, the blue-staining fibers stand out in sharp contrast to the other, orange-staining, constituents of the cytoplasm. The starch mass continues to be a conspicuous feature of the cell; in the cell represented in Figure 7, most of it lies above the plane of the drawing.

As the chromosomes contract further and the nucleus goes into a well-marked diakinesis stage, the cone-shaped groups in the cytoplasm are even less in evidence. The fibers are most closely packed in a region about halfway between the nuclear and plasma membranes; from this denser region they extend in to the nuclear membrane, their outer ends converging in small groups here and there (Fig. 8), but there is little suggestion of the arrangement as found in the earlier "bipolar" stages. The fibers are fairly uniformly distributed about the nucleus excepting in the region occupied by the starch mass. Here they are much fewer in number, and at times, apparently at least, altogether wanting. The section represented in Figure 8 gives a good view of the relations of the various parts of the cell at this time; it passes approximately through the center of the starch mass and a little below the center of the nucleus. The starch grains are numerous and frequently aggregated in small groups, commonly, although not invariably, four in a group. The apparently finely granular material between the grains now tends to take a reddish to an orange stain. The nucleus still contains numerous nucleoles.

Usually it is difficult to trace the nuclear membrane in the region where the starch mass is in contact with it. This is true of the stages as early as those represented in Figures 5 and 6. The appearance of the preparation at times suggests that the nuclear membrane may be breaking down at that point;

¹⁵ Osterhout, W. J. V.: Spindle formation in *Agave*. *Proc. Cal. Acad. Sci.*, 3rd ser., Bot., ii (1902): 262, Pl. XXVI, Figs. 9-11, also Pl. XXV.

it seems probable, however, that the difficulty in distinguishing the nuclear membrane here is due to the over- and underlying dense starch mass; at any rate the starch mass will give such an appearance as is found even if the nuclear membrane is of uniform thickness throughout.

The fact that the spore mother-cells of *Marsilia* show a distinct polar organization from an early period in their development, makes it seem likely that the forming spindle should be bipolar from the beginning of its appearance, an expectation which is increased by the early appearance of a bipolar arrangement of the fiber groups in the cytoplasm. A study of the spore mother-cells at the time when the nuclear membrane has just disappeared shows, however, that apparently this expectation is not realized. The spindle fibers, which by that time have assumed the smooth, clean-cut appearance characteristic of the fibers as found in the completed spindle, are not arranged in a bipolar figure, but in several perfectly distinct and separate poles. The spore mother-cells of *Marsilia* show as striking "multipolar" spindles as are found anywhere. Figures 9a and 9b represent two consecutive sections of a spore mother-cell with four well-developed poles; 9a lies above 9b, and the starch mass lies in the section above that from which 9a is taken. Fibers from each of the four poles run up to the chromosomes, and in addition there are some running more or less continuously from one pole to another; this is to be seen in the two lower poles of the cell figured. There are also some fibers connecting the other poles, but they are not so numerous nor so conspicuous. The idea suggests itself, but it is only a suggestion, that the two poles conspicuously connected by fibers will be the definitive spindle poles. One of the four poles lies in close contact with the plasma membrane, two others have at least some fibers running up to the plasma membrane, and the fourth one lies distinctly free from it, although it comes close to it. That this pole is not in contact with the plasma membrane in a plane above or below that of the drawing can be easily determined by focusing up and down.

It is a fact worth noting that many of the "multipolar" spindles which have been figured are quadri- and tripolar

spindles (the latter possibly also, in many cases at least, really quadripolar); furthermore, as has frequently been pointed out, multipolar spindles have been described so far practically only for spore mother-cells. To mention a few instances, Berghs figures a roughly tripolar spindle for *Narthecium ossifragum*,¹⁶ Grégoire and Berghs figure several for *Pellia epiphylla*,¹⁷ and Miyake several for *Galtonia candicans*,¹⁸ and one for *Tradescantia virginica*.¹⁹ It would be interesting to know in how many cases the spindle of the first division in spore mother-cells starts as a quadripolar figure. It seems that good multipolar stages are difficult to find in most plants.²⁰ Frequently cells figured for such stages show evidence of poor fixation. It is a common experience that the multipolar stage is difficult to fix well. All this merely emphasizes the necessity of caution in determining whether there is in general any indication that the spindle for the first division in spore mother-cells starts as a quadripolar figure.

Unfortunately, the stages intervening between the disappearance of the nuclear membrane and the establishment of the bipolar spindle appear to be passed over rapidly in *Marsilia*, so that they are only occasionally found in the fixed material, and then it seems that they are difficult to fix well; at any rate, I have found very few satisfactory preparations of this stage. Strasburger does not figure any stages between diakinesis with the nuclear membrane intact and the practically completed bipolar spindle; he mentions a multipolar stage for *M. quadri-folia*,²¹ but the section represented in his figure shows only two poles. In his Figure 86 Strasburger represents a spore mother-cell of *M. Drummondii* in diakinesis with two well-developed spindle poles. It is perhaps a question, however, whether these represent the definitive spindle poles.

¹⁶ *La Cellule*, xxii, Pl. I, Fig. 23.

¹⁷ *La Cellule*, xxi, Pl. II, Figs. 1 and 2.

¹⁸ *Jahr. f. wiss. Bot.*, xlii, Pl. III.

¹⁹ *Ibid.*, Pl. V.

²⁰ Mottier figures excellent ones for *Lilium* and *Podophyllum* (*Jahr. f. wiss. Bot.*, xxx, Pls. III and IV, and xxxi, Pl. II, Fig. 4), and Osterhout for *Equisetum* (*Jahr. f. wiss. Bot.*, xxx, Pl. I).

²¹ *L. c.*, p. 146; Fig. 60.

The chromosomes at the stage represented by Figure 9 are crowded near the central part of the cell, a condition which suggests a pushing in on them on the part of the spindle fibers. In this connection it should also be mentioned that at one stage shortly after synapsis, when the chromatin strands are long and of fairly uniform diameter, they apparently lie in a loose tangle on the side of the nucleus opposite that at which the synaptic mass was located (Figs. 5 and 6). It looks as though the chromatin strands were pushed over to that side, although the cytoplasmic fibers cannot be traced at this stage into the nuclear cavity.

As the nuclear membrane disappears during diakinesis and the spindle for the first division forms, the starch mass apparently continues to occupy the same position in the cell that it had during the earlier stages. In presynapsis and early synapsis stages, the starch mass invariably lies on the outer side of the cell; later it is frequently found on the side nearest the interior of the spore sac or in some intermediate position. It is to be noted, however, that at just about this time the spore mother-cells commence to round off and separate more or less from each other, and it seems that at this time they rotate more or less so that their original position is changed. At another time I hope to enter more into detail upon the motions which the spore mother-cells undergo at this period; suffice it for our present purposes to say that, as far as my data go at present, they do not indicate that the starch mass undergoes any translocations within the cell up to the time of the first nuclear division.

The spindle figure for the first division is unusually sharply defined. The fibers are delicate, numerous, and take a brilliant blue with the triple stain; they are sharply differentiated, so that they are readily followed in cross sections of the spindle. The poles are often rather broad and lie close to the plasma membrane, although they do not necessarily extend to it. The fibers twist about each other and frequently come together in little groups at their polar ends, and often a small, deeply staining granule lies at the end of such a group. There seems to be no constancy in the number of such groups in a spindle.

Distinct fibers running continuously from pole to pole can be easily distinguished between others, often considerably heavier, which end at the chromosomes (Figs. 10 and 11). In the metaphases, the spindle at times shows a peculiar zonated appearance, a dark band running across it in one or two places between the equatorial plate and the poles. These dark bands are symmetrically placed in the two halves of the spindle and seem to be due to a thickening of the fibers at these regions. This suggests the possibility that in the living cell there may be waves of contraction passing over the spindle. The fact that such zonated spindles are not often found in fixed material does not argue strongly against this possibility, for it is only under an exceptionally favorable combination of circumstances that the fixation of such "waves" could be expected. A more important argument against this possibility lies in the fact that in studies which have been made on karyokinetic figures in living cells nothing has been observed, as far as I am aware, of waves of contraction passing over the spindle. The difficulty of observation here, however, discounts the conclusiveness of a negative result.

The chromosomes on any spindle show considerable differences between themselves both in form and size, differences which are, if anything, even more marked in the late diakinesis stages and shortly after the breaking down of the nuclear membrane. Equally remarkable is what may be called a physiological difference between the chromosomes. There are regularly found around the periphery of the spindle small chromosomes whose halves separate considerably earlier than those of the other chromosomes, so that they are halfway to the poles when the halves of the others are still together at the equator of the spindle. It seems that for my specimens of *M. quadrifolia* this is a regular occurrence. Strasburger observed that in the equatorial plate stages of *M. drummondii* a varying number of chromosome pairs separate before the others; but he finds this associated with a more or less abnormal course of division, and refers it to a diminished attraction between homologous chromosomes. In its extreme case, this diminution in

the attraction between homologous chromosomes gives rise to diploid figures.²²

It is to be noted that the axis of the spindle figure lies at right angles to the original main axis of the cell. The starch lies as a more or less flattened mass of oval outline to one side of the spindle. Rarely do any of the starch grains lie among the spindle fibers, or spindle fibers extend into the region of the starch mass. As the chromosomes collect at the poles and the daughter nuclei are reconstructed, a conspicuous system of connecting fibers develops between them. The fibers are heaviest at the middle and become more delicate and interlaced as they approach the nuclei. In addition to these connecting fibers, there is a more or less well developed system of polar radiations extending out from the region of the nuclei; as a rule these radiations are not conspicuous in the first division.

During, or somewhat previous to, the telophases, definite and characteristic changes occur in the position of the starch mass. It gradually moves in between the two daughter nuclei. In so doing it passes around the periphery of the spindle faster than through the spindle itself, and as a result the starch mass is for a time roughly horse-shoe-shaped. In the cell represented in Figure 12, the starch extends almost completely across the cell in the planes above and below the spindle, but in the plane of the spindle itself it is not halfway across. As the starch mass is coming in between the spindle fibers, the latter are disappearing. As is well known, cell division does not follow the first nuclear division in the spore mother-cells of *Marsilia*. The connecting fibers, however, frequently persist for a considerable period, and it is not uncommon to be able to trace them between the starch grains. The starch mass finally forms a relatively thin plate lying midway between the two daughter nuclei and extending almost to the periphery of the cell. It is to be noted that its plane is now at right angles to the position it occupied at the time of the equatorial plate stage and earlier.

²²L. c., pp. 152, 155; Figs. 90, 102.

The chromatin of the reconstructed daughter nuclei is often largely confined to the periphery of the nucleus, and nucleoles reappear. In the prophases of the second division there is a conspicuous development of fibers, and in this division it is not difficult to find pronounced multipolar stages in the formation of the spindle. The fibers are again characterized by an unusually clean-cut appearance; they envelop the chromosomes in a dense tangle, and at their distal ends they come together in numerous places to form sharply pointed poles, each of which consists of relatively few fibers (Fig. 13). Frequently the poles are somewhat curved. The upper spindle of Figure 13 is cut to one side, most of the chromosomes lying in the next section. The completed spindle again is a well defined figure. It is more slender than that of the first division, and the poles tend to be somewhat sharper, but the individual fibers stand out with the same distinctness and take the same brilliant stain as in the first division.

The orientation of the spindles of the second division shows some interesting variations; as is frequently the case in the second division in spore mother-cells, the two spindles lie at various angles to each other. In some cases the two spindles and the starch mass lie in three parallel planes. The axes of the spindles may then be parallel to each other, at right angles to each other, or at some intermediate angle. In other cases, however, the plane of one or both of the spindles is at an angle to that of the starch mass, and then it frequently happens that the plate-shaped starch mass is somewhat deformed. Figures 14*a* and 14*b* represent two successive sections of such a cell; 14*a* lies above 14*b*. The lower spindle lies in the plane of the section, and ordinarily the starch mass would lie some distance above it, also in the plane of the section. In this case, however, the upper spindle is at an angle to the plane of the section, the right hand pole lying lower, the left hand one higher, and the starch plate has been depressed on the right hand side of the cell. The starch plate apparently continues to form a rather complete separation between the spindle-figures of the second division until late in the anaphases of that division.

As the chromosomes move back to the poles, the polar ends of the spindle become markedly darker and more compact (Fig. 14*b*). This stands out even more strikingly in the preparations than in the drawing, where only a section of the spindle is represented. The spindle shown in Figure 14*a* is essentially of the same appearance as that in Figure 14*b*; the right hand pole, however, is represented lighter because it lies deeper in the section; the left hand (higher) pole, on the other hand, has a large proportion of its fibers in the next section above, so that it does not appear heavy in the drawing. The spindle figures at this stage show well-developed connecting fibers running from pole to pole, and it can readily be seen that some of them lie distinctly outside of the chromosome group. Delicate radiations extend out from the poles of the spindle towards the equatorial region; at times radiations from the two poles cross in the equatorial region. Although delicate, these fibers because of their distinct blue stain stand out sharply in the preparations against the orange-gray background of the other cytoplasmic structures. The poles of the spindles frequently do not reach to the plasma membrane. Occasionally, in what otherwise seem to be somewhat abnormal cells, the spindle poles are unusually sharp and compact and end directly at the plasma membrane; if the cells are somewhat shrunken, the plasma membrane is markedly pushed out at the points where the spindle poles are in contact with it. More or less curved spindles also are not rare.

After the chromosomes reach the poles and the daughter nuclei are reconstructed, the starch is so distributed that about an equal amount is contained in each of the four cells resulting from the development of cell-plates by means of the connecting fibers between the nuclei.

The spore mother-cells of *Marsilia* show for a considerable period of their existence a well-marked polar organization. In the earlier stages, the visible expression of this as far as the cytoplasm is concerned is the characteristic position of the starch grains in the cell. There is no evidence of their being enclosed by an enveloping membrane as is the case in young

leaf cells of *Isoetes*.²³ Later, during the processes of nuclear division in the spore mother-cells of *Marsilia*, as has been described, the starch grains undergo definite and specific translocations; but these translocations are conspicuously different from those of the starch grains in young vegetative cells of *Isoetes*. This difference is at least partly due to the fact that in these cells of *Isoetes* the starch grains are confined to the interior of the "polar structure," and hence necessarily change their position as its position changes. The cells of *Marsilia* apparently do not possess a structure like the "polar structure" of *Isoetes*. It is a striking fact that in *Marsilia* the often numerous starch grains, although as far as apparent not enclosed in any kind of an enveloping membrane, remain fairly closely packed in a single group from soon after the time of their first appearance in the cell until the completion of the second division; and we have in the specific changes of position and form which this group of starch grains undergoes an interesting point of attack upon the problem of the forces operating in the cell during nuclear division. Starch grains, however, do not universally show in their position and movements a marked effect of the forces operating in the cell during nuclear division. The figures of Grégoire and Berghs for the early divisions in the germinating spore and in the spore mother-cells of *Pellia epiphylla*²⁴ are of interest in this connection. There the starch grains are apparently scattered quite irregularly throughout the cytoplasm (not, however, through the karyokinetic figure).

Contrary to what might be expected, the polar organization of the spore mother-cells of *Marsilia*, which is so conspicuous during the earlier stages, seems to have quite disappeared at about the time when the nuclear membrane breaks down, or at any rate it seems to find no expression in the arrangement of the spindle fibers just preceding the formation of the bipolar spin-

²³ Marquette, W.: Manifestations of polarity in plant cells which apparently are without centrosomes. (*Beih. bot. Centralbl.*, xxi : 281. 1907).

²⁴ Grégoire, V., and Berghs, J.: La figure achromatique dans le *Pellia epiphylla*. *La Cellule*, xxi : 193. 1904.

dle. It will be necessary to have more extensive data on the orientation and position of the fiber groups at this stage before the relation of the "multipolar" (or quadripolar) spindle to the earlier organization of the cell can be advantageously discussed.

It is as yet impossible to judge whether in general a multipolar origin of the spindle is to be regarded as inconsistent with a permanent polar organization of a cell. Careful study may show that even during the multipolar stages, where they occur, the original polar organization of the cell is maintained, and that the cell-axes can be traced continuously.

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EXPLANATION OF PLATE VIII.

The figures of this and the following plate were drawn with the aid of the camera lucida, the magnification about 1,700 diameters in each case. All figures are from spore mother-cells of *Marsilia quadrifolia*.

Fig. 1. Young spore mother-cell, showing characteristic aggregation of chromatin at central part of nucleus.

Fig. 2. Somewhat older spore mother-cell, showing an elongated, vacuolar region in the cytoplasm at *a*.

Fig. 3. An approximately full-sized cell; aggregation of starch grains on the side of the nucleus facing the sporange wall.

Fig. 4. Synapsis; the chromatin strands oriented on a nucleole lying close to the nuclear membrane.

Fig. 5. Post-synapsis: the chromatin strands lying on the side of the nucleus opposite that occupied during synapsis. A fairly well-developed bipolar figure of fibers in the cytoplasm, an indication of a third pole at the lower left corner. Most of the starch mass is in the next section.

Fig. 6. Two cells in approximately the same stage as the preceding. In one of them a granule simulating a "centrosome."

Fig. 7. A later stage, showing unsymmetrical arrangement of the cytoplasmic fibers; a number of cone-shaped groups lie at the lower left corner of the cell below those shown in the figure. A considerable number of starch grains lie above the plane of the drawing.

Fig. 8. Diakinesis.

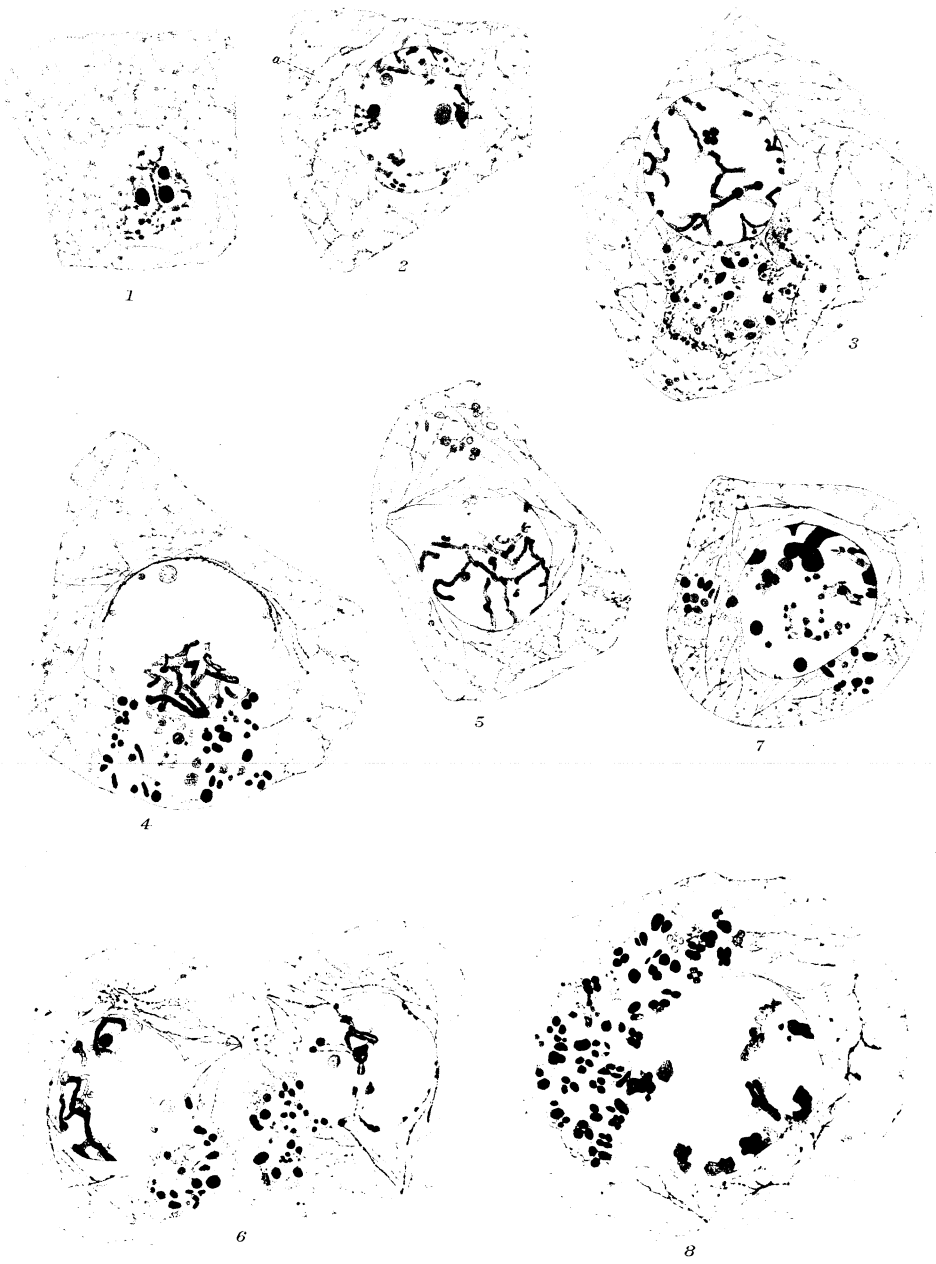


PLATE IX.

EXPLANATION OF PLATE IX.

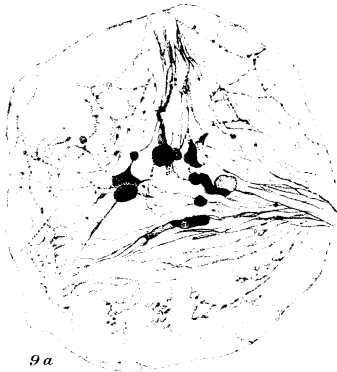
Figs. 9 *a, b*. Two successive sections of a cell showing a quadri-polar spindle. 9*a* lies above 9*b*, and the starch mass is in the section above 9*a*.

Figs. 10 and 11. Completed spindle. Some chromosomes are well on the way to the poles, while others are still at the equator of the spindle.

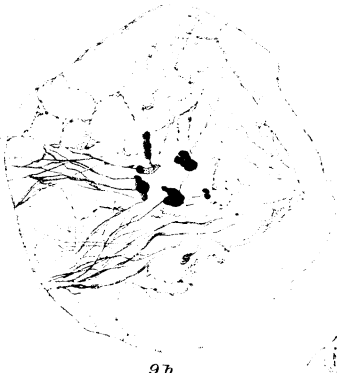
Fig. 12. Reconstructed daughter nuclei. The starch mass moving in between the nuclei; in the planes above and below that of the drawing it reaches almost completely across the cell.

Fig. 13. Multipolar stage in the formation of the spindles in the second division. The starch mass forms a plate across the cell separating the two figures.

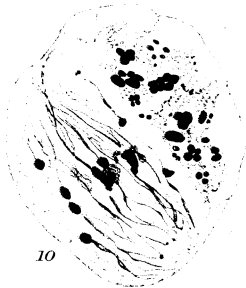
Figs. 14 *a, b*. Two successive sections of cell in anaphases of second division, 14*a* above 14*b*. The spindle represented in 14*a* lies at an angle to the plane of the section, the left hand pole being higher (part of it lies in next section above), the right hand pole lower and hence represented lighter. The left pole is above the starch plate. The starch plate is depressed by the right pole and appears in part in Figure 14*b*. The spindle shown in 14*b* lies in the plane of the section. The spindle poles of 14*a* are as well developed as those of 14*b*.



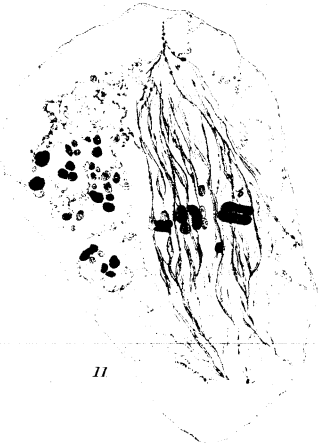
9a



9b



10



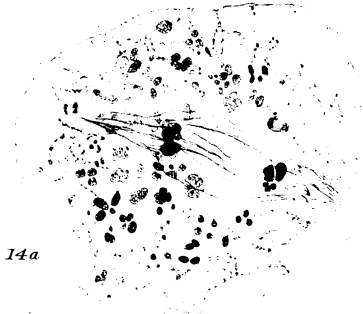
11



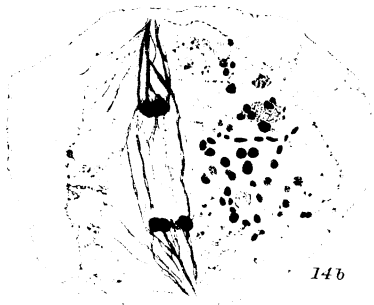
12



13



14a



14b

