

is suggested that the cell membrane or cytoplasm is constituted by a heterogeneous system of lipoids, proteins, etc., held together in a magma containing a gel-forming substance with physical properties similar to those of the cholates. On such a hypothesis, the biological action of certain substances can be explained in a manner more satisfactory than is possible by the assumption of the "lipoid" theory of Hans Meyer and Overton.

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*Gametogenesis and Sex-Determination in the Gall-Fly, Neuroterus lenticularis (Spathogaster baccarum).—Part III.*

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[PLATES 6 AND 7.]

In two previous papers on the maturation of the eggs and spermatozoa and determination of sex in *Neuroterus lenticularis*\* I showed (1) that any individual female of the agamic generation produces either male or female offspring, but not both; (2) that the eggs of some agamic females undergo a reduction division at maturation, while those of others do not; (3) that, since males have 10 chromosomes in the germ-cells before maturation, while females have 20, the eggs which undergo reduction produce males, and those which do not, produce females; (4) all eggs of the sexual generation undergo a double maturation division, and are fertilised, giving rise to females of the agamic generation with 20 chromosomes in the ovarian cells. The maturation divisions in the eggs of the sexual generation were described as of a rather peculiar type, and some difficulty was experienced in forming a clear and connected idea of the process.

One important problem remained unsolved—the nature of the difference between the male-producing and female-producing females of the agamic generation. No difference was discernible between the flies of the two types, nor could any difference be found between their chromosome-groups in the ovarian divisions. A tentative suggestion was made that the difference might depend on the existence of two kinds of spermatozoa, one of which might cause the fertilised egg to develop into a male-producing, the other into a female-producing agamic female, and this suggestion seemed to gain

\* 'Roy. Soc. Proc.,' B, vol. 82, p. 88 (1910); and B, vol. 83, p. 476 (1911).

some support from the fact that in the spermatogenesis an extra-nuclear body was observed not to divide at the single spermatocyte division, but to pass over into one of the two spermatids.

Further investigation of the matter was interrupted by other work until 1913, but in that year I determined to test the hypothesis mentioned by rearing the offspring of individual sexual females separately, and then finding whether each family contained both male-producing and female-producing individuals. If they did not, that is to say, if the offspring of any one sexual female were all male-producing or all female-producing, the hypothesis that the difference depended on two kinds of spermatozoa would be disproved, for the receptaculum seminis of the fertilised female should contain both sorts of spermatozoa, and therefore both kinds of fertilised eggs should be laid by the one female.\* At the same time I determined to test afresh the possibility that the two kinds of agamic females were derived respectively from fertilised and parthenogenetic eggs laid by the sexual females. This seemed very improbable, for in almost every egg of the sexual generation examined for the maturation divisions, a spermatozoon was found, and it was therefore almost certain that all the eggs of the sexual generation are normally fertilised.

For the purpose of these experiments a number of females of the sexual generation were sleeved on oak twigs in May, 1913, one female in each sleeve. Some of the females used had been seen to copulate with males; in other cases two or three males were enclosed in the sleeve with the female so that fertilisation would almost certainly take place, and finally a number of virgin females, from galls kept separately so as to make the access of a male impossible, were also sleeved on other twigs of the same oaks. In several instances virgin females laid eggs in the leaves, but in no case was a gall produced, and the eggs appear either not to have developed at all or to have died at a very early age. It may, therefore, be taken as certain that females of the sexual generation do not reproduce parthenogenetically. Of the fertilised females, about a dozen produced galls on the leaves, some of them in considerable numbers. These galls were allowed to grow until ripe in October, then collected and kept separately through the winter. In February and early March a number of branches of an oak tree were covered with large muslin sleeves to prevent the access of wild flies, and when the galls were about to hatch they were placed in sleeves on the branches which had been covered, and also in some cases on uncovered branches, since the

\* If there were two kinds of males, producing different spermatozoa, the result under consideration would still be obtained if each sexual female paired with only one male. This hypothesis is further considered later.

supply of galls was more than enough for the sleeves on the branches which had been covered. About four to eight galls were put in each sleeve, and since some of the parent flies had produced 50-60 galls, in some cases a dozen or more sleeves were used for the galls produced by a single sexual female in the previous summer. In all, over 80 lots of galls were sleeved out. It would probably have been better, in some cases at least, to put only one gall in each sleeve, but as my previous experience had shown that flies when sleeved singly frequently fail to produce any galls, I judged it better to put several galls in each sleeve in order to be more certain of getting a result. As it happened, the season was extraordinarily favourable, and when the leaves appeared those inside the sleeves were crowded with galls. Not quite all were collected, but I hatched and noted the sex of about 9000 flies, as shown in the Table on pp. 186 and 187.\*

Inspection of this Table shows at once that the vast majority of flies reared in the sleeves containing galls produced by any one sexual female are of one sex, so that the sexual females A, B, F, H, J, and L had male-producing daughters, while the others had female-producing. In most families, however, there is a small percentage of exceptions, and the question arises, what is the explanation of these? If they occurred exclusively in the sleeves on branches which had not been covered before the galls were sleeved out, it might be assumed that they were produced by very early wild flies which had laid eggs in the buds before the sleeves were put on the tree. They are, however, about as numerous in sleeves on branches which had been covered as on uncovered branches. Further it is to be noticed that the exceptional males in female families are about as numerous as females in male families, and that the number of exceptions varies largely from sleeve to sleeve in the same family. For example, among the flies reared from sexual female C, sleeves 22*a*, 23, 24, 24*a*, 25, 27*a* had no exceptions, with a total of 873 females, while sleeves 21, 22, 27, 28, 29 had a total of 501 females and 33 males, or over 6 per cent. of exceptions. The other families show the same sort of thing. In all, of 80 sleeves (35 male-producing, 45 female-producing), 18 of the male sleeves, containing 1,543 males, and 24 female sleeves, containing 2,561 females, had no exceptions. Thus almost exactly half the sleeves were without exceptions, and these sleeves were evenly divided between the male and female producing, and contained nearly half the total flies reared (4,014 out of 9,574). Frequently, also, the exceptions appeared among the later flies to hatch; this was only definitely recorded in the case

\* I take this opportunity of recording my indebtedness to Mr. F. Balfour Browne for his kindness in preserving some of the flies as they hatched, while I was prevented by indisposition from attending to them.

Reference Numbers of Sleeves, above (those which were on branches which had been previously covered *italicised*).  
Numbers and Sexes of Flies Hatched in each Sleeve, below.

Sexual female parents.	Totals.													
A. Galls sleeved, March 2 ...	<i>1</i> 28 ♂	<i>5</i> 113 ♂	<i>7</i> 71 ♂	—	—	—	—	—	—	—	—	—	—	212 ♂
B. Galls sleeved, March 2 ...	<i>10</i> 209 ♂ 2 ♀	<i>14</i> 39 ♂ 2 ♀	<i>14A</i> 130 ♂ 28 ♀	15 106 ♂ 1 ♀	15A 58 ♂ —	16 136 ♂ —	18 119 ♂ 2 ♀	18A 42 ♂ —	—	—	—	—	—	839 ♂ 35 ♀
C. Galls sleeved, March 10...	<i>21</i> 15 ♂ 107 ♀	<i>22</i> 2 ♂ 38 ♀	<i>22A</i> — 179 ♀	23 — 143 ♀	24 — 86 ♀	24A — 154 ♀	25 — 199 ♀	27 1 ♂ 22 ♀	27A — 112 ♀	28 8 ♂ 54 ♀	29 7 ♂ 280 ♀	—	—	33 ♂ 1374 ♀
D. Galls sleeved, March 10...	<i>30</i> — 139 ♀	<i>31</i> — 73 ♀	<i>31A</i> 1 ♂ 202 ♀	32 — 82 ♀	33 — 318 ♀	34 3 ♂ 118 ♀	35 4 ♂ 181 ♀	37 6 ♂ 197 ♀	38 2 ♂ 103 ♀	38A — 66 ♀	39 1 ♂ 71 ♀	39A 7 ♂ 133 ♀	—	24 ♂ 1683 ♀
E. Galls sleeved, March 17...	<i>40</i> 35 ♀	—	—	—	—	—	—	—	—	—	—	—	—	35 ♀
F. Galls sleeved, March 11...	<i>41</i> 259 ♂ 1 ♀	<i>42</i> 267 ♂ 4 ♂	<i>42A</i> 186 ♂ —	43 192 ♂ 2 ♀	44 70 ♂ —	45 79 ♂ 3 ♀	46 27 ♂ 3 ♀	47 23 ♂ —	—	—	—	—	—	1108 ♂ 13 ♀

G. Galls sleeved, March 11...	51 — 54 ♀	51A — 262 ♀	52 7 ♂* 232 ♀	53 — 115 ♀	54 — 77 ♀	54A 9 ♂ 82 ♀	55 — 125 ♀	56 1 ♂ 128 ♀	57 — 18 ♀	58 — 12 ♀	59 23 ♂ 79 ♀	— —	40 ♂ 1184 ♀
H. Galls sleeved, March 11...	60 66 ♂ —	61 51 ♂ —	62 53 ♂ —	63 81 ♂ —	64 92 ♂ 5 ♀	68 109 ♂ —	69 243 ♂ 2 ♀	— —	— —	— —	— —	— —	695 ♂ 7 ♀
I. Galls sleeved, March 17...	71 — 59 ♀	72 1 ♂ 95 ♀	73 — 122 ♀	74 1 ♂ 67 ♀	75 — 25 ♀	76 12 ♂ 87 ♀	77 — 42 ♀	78 — 64 ♀	79 3 ♂ 189 ♀	— —	— —	— —	17 ♂ 760 ♀
J. Galls sleeved, March 17...	80 176 ♂ —	81 30 ♂ —	82 102 ♂ 2 ♀	83 39 ♂ —	84 322 ♂ 5 ♀	85 82 ♂ 1 ♀	86 241 ♂ 2 ♀	87 211 ♂ —	— —	— —	— —	— —	1208 ♂ 10 ♀
K. Galls sleeved, March 17...	110 3 ♂ 113 ♀	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	3 ♂ 113 ♀
L. Galls sleeved, March 17...	125 183 ♂ 18 ♀	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	183 ♂ 18 ♀
Total offspring of females A, B, F, H, J, L, 4235 ♂, 83 ♀ (= 1·92 per cent. ♀). Total offspring of females C, D, E, G, I, K, 5139 ♀, 117 ♂ (= 2·23 per cent. ♂). Grand total, 9374, of which 200 (2·09 per cent.) are exceptions.													

\* The 7 males in No. 52 all hatched after most of the females.

of Series G, sleeve 52, but it certainly occurred in other cases also. These facts strongly suggested that the exceptions were due to the presence of eggs of wild flies, which were remarkably numerous in 1914. It seemed probable that, as the buds on the twigs of necessity pressed against the muslin in some of the sleeves, wild flies might have been able to insert their ovipositors through the meshes of the muslin and lay eggs in such buds. The sleeves used were of muslin of two kinds, one considerably finer than the other. Unfortunately no record was kept of which galls came out of sleeves of the two grades of coarseness, but it seems probable, if the explanation suggested is correct, that the sleeves containing no exceptions were of the finer mesh. I was prevented in the summer of 1914 from repeating the whole experiment, as I wished to do, and an attempt made in 1915 failed because the flies laid no eggs; but, in order to test whether flies can lay through the meshes of muslin, in the spring of 1915 I sleeved a number of twigs, and then put a number of galls in larger sleeves fastened outside the others. Some sleeves were also left on the twigs with no outer sleeve, to see whether galls would be produced in them by wild flies. As this was done before the end of February, two or three weeks before the flies begin to emerge, there is no chance of eggs having been laid in the buds before the sleeves were put on. The result of this experiment was that out of 19 sleeves, each with galls in an outer sleeve enclosing it, three contained galls of the sexual generation in May. In each case the galls were on leaves derived from one bud only; in one sleeve there were 20, in the second 18, in the third 2. From the first two there were reared 17 females in the one case, 15 females and 2 males in the other.

Five twigs were covered with simple sleeves, to see whether galls might be produced in them by wild flies, but none contained any. Although a much smaller proportion of galls were produced inside the sleeves than in the preceding year, this experiment proves conclusively that it is possible for the flies to oviposit through the meshes of the sleeves used, and the smaller proportion is probably due to the fact that the season of 1915 was much less favourable to the flies than that of 1914. Wild galls were extremely scarce at Cambridge as contrasted with their great abundance in 1914, so that if it had not been for the kindness of my friend Mr. E. E. Unwin, who sent me a supply of galls from Reading, I might have not been able to obtain enough to supply sufficient material to complete the cytological work described below. It is probable that the frost and snow of the last days of March, following a mild early spring, killed many of the flies before they had laid. I think it may, therefore, be concluded with certainty that the exceptions recorded in about half the sleeves in the 1914 experiments were due to eggs laid by wild flies.

*Maturation of the Fertilised Eggs.*

The results of the experiments described make it certain that any individual sexual female produces exclusively, or almost exclusively, male-producing or female-producing agamic offspring. The experiments, therefore, disprove the hypothesis that the two types of agamic female are due to dimorphism of the spermatozoa produced by one male. Two possibilities then remain: the two types of agamic female may be due to two kinds of eggs laid by different sexual females; or, if each sexual female mates with only one male, they may be due to two kinds of males which produce different spermatozoa, as has been suggested by Morgan in the somewhat analogous case of *Phylloxera caryocaulis*.<sup>\*</sup> I have carefully re-examined my preparations of the spermatogenesis in a number of males, and can find no regular differences among them; while not definitely denying their existence, I am unable to find any evidence for them. There is certainly no difference in chromosome number, nor can I find that any chromosome is constantly larger or smaller in some males than in others. It remained, therefore, to discover whether any differences could be found among the eggs laid by different sexual females, and on the analogy of the facts described in my earlier papers on the subject, it seemed possible that such differences might be expected in the maturation divisions. Some indication that such differences might exist was given in the first paper, but as there was then no suspicion that the females were of two types, the eggs of individual females were not preserved separately. It was therefore necessary to collect fresh material. For this purpose, in the spring of 1914, and again in 1915, a number of females were allowed to lay separately, and their eggs preserved in Gilson's acetic alcohol sublimate (absolute alcohol, glacial acetic acid and chloroform, equal volumes; sublimate to saturation) at various ages up to about four or five hours after laying. I have cut sections of these eggs (I wish to record my indebtedness to Mr. D. W. Cutler for valuable help in this part of the work) and have also re-examined all the eggs preserved in 1906 and 1907, on which the description in my previous paper was based. The account that follows is thus derived from a series of over 300 eggs of 15 separate females, in addition to about 200 selected eggs of mixed females. I estimate, however, that fully half of these are in stages either too young or too old to throw any considerable light on the maturation process.

The first suggestion of a difference in the maturation process of eggs of different females was that in some eggs the polar chromosomes, after the

<sup>\*</sup> T. H. Morgan, "The Predetermination of Sex in Phylloxerans and Aphids," 'Journ. Exp. Zool.,' vol. 19, p. 285 (1915).

completion of the maturation divisions, remain separate and distinct, in some cases until the second or third segmentation division of the zygote nucleus, while in other eggs they become clumped together quite early, and cease to be distinguishable in the segmentation divisions. To some extent this difference does appear to be characteristic of the eggs of some females, but it is by no means regular, and I think it cannot be regarded as a really distinguishing feature.

A second difference in the maturation of eggs laid by different females is found at an earlier stage, but it is difficult to say whether it is really significant. In all cases the nucleus before maturation comes to the surface of the egg, when it is small and evenly stained, and is usually, perhaps always, flattened for a time against the edge of the egg.\* The nucleus then enlarges, and its subsequent behaviour seems to show differences in the eggs of different females. The differences between the two types will be described briefly, before giving a more detailed account of the maturation processes. In the eggs of some females it becomes top-shaped, or like two cones attached to each other by their bases, the points of the cones being in a line perpendicular to the surface of the egg (Plate 6, Figs. I, 1 and 2; II, 1 and 2; III, 1). As the nucleus assumes this form, the chromatin becomes concentrated chiefly at the inner and outer points, but there is often also a single or double ring of granules round the equator, or common base of the two cones, which give the appearance of chromatin emitted from the nucleus. In the subsequent stages of these eggs, as will be described more fully below, the separation of the chromosomes which will form the egg-nucleus from the inner polar chromosomes occurs almost simultaneously with the division of the outer group of polar chromosomes, with the result that as a rule three quite distinct groups of polar chromosomes are formed (Figs. III, 7; IV, 5 and 6).

In eggs of other females the process seems to be slightly different. The nucleus appears never to assume the top-shaped form, but swells up into a spherical nucleus containing a well marked reticulum, sometimes with larger aggregations of chromatin at the outer side or round its equator (Plate 7, Figs. VII, 1; VIII, 1). In the later stages of these eggs the separation of the innermost group of chromosomes, which sink in to form the egg nucleus, appears to take place considerably before the division of the outer group, and the latter division is apparently often incomplete, so that only two groups of polar chromosomes may be found in the later stages.

It is not difficult to choose series of eggs of individual females (*e.g.*, Nos. III and VII, Plates 6 and 7) in which the differences described are fairly pronounced, but on the other hand eggs of other females show figures

\* Cf. Part II of this series, 'Roy. Soc. Proc.,' B, vol. 83, Plate 17, fig. 3.

which it is not easy to place in either group with complete confidence. For example, the eggs shown in Series VI, 1-7 (Plate 7), are intermediate in their early stages between the top-shaped and spherical nuclei, and the later stages, though not very clear, might belong to either type. Similarly, while the early stages of the series shown in Figs. I, 1, 2, are clearly of the top-shaped type, the later figures (I, 3 and 4) might well belong to the second type. While, therefore, there are some indications of two distinct types of maturation process in the eggs of different females, the results obtained do not make these differences so certain as to justify any confident conclusion that they correspond with the male-producing and female-producing offspring.

Whether the differences noticed in the eggs laid by different females are connected with the fact that the flies to which they give rise are either male-producing or female-producing may be doubted, but in any case the nature of the nuclear divisions during maturation is so remarkable as to need a somewhat fuller description. After the nucleus has swollen up, whether it be of the "top-shaped" or "spherical" type, its membrane disappears, usually first at the inner pole (Fig. VIII, 1), and from the nuclear reticulum fine strands are drawn out towards the centre of the egg. Some figures suggest that these are at first loops, but others (*e.g.*, Figs. VIII, 1; IX, 1-6) that they are strands of chromatin not connected with each other at their inner ends. Many figures show that these strands are double at their bases (IV, 3; VII, 4, 5, 6; IX, 1, 2, 3, 4), and when, as not infrequently happens, they appear to arise singly from small masses of chromatin (III, 4; IV, 2; VII, 2), it is possible that these masses are formed by coalescence of two threads in consequence of somewhat defective preservation. In good figures it is usually clear that these strands are about 10 in number, and when only a smaller number is visible it is possibly because some are longer than others, and only the long ones are sufficiently clear of the reticulum to be recognised. While these strands are being formed at the inner side of the nucleus similar but shorter rods or loops are formed also at the opposite side, towards the edge of the egg, and the two series are for a time connected by a network, on which the chromatin is sometimes aggregated into deeply-stained masses.

The subsequent behaviour is extremely hard to follow, and is possibly different in the two classes of eggs, the existence of which has been suggested above. What appears to happen is that the reticulum between the inner and outer rods disappears, perhaps by becoming concentrated into small chromatin masses attached to the proximal ends of the strands (Figs. III, 4; IV, 4), and the inner group of strands then separates itself from the outer

and sinks for a short distance into the egg. The two groups so formed may be temporarily connected by strands of chromatin (Figs. X, 1 and 2). A system of spindle-fibres can then be seen running inwards from the inner ends of the strands, and also, but less clearly, outwards from these strands towards the outer group; in at least one case (Fig. II, 4) the inner spindle-fibres can be seen to be 10 in number. At this stage the strands of the inner group lie as a rule fairly accurately parallel with one another, but show a tendency to become clumped, so that they commonly have the appearance of less than ten moderately thick rods (Figs. I, 3; II, 4; III, 5, 6; VI, 7). The outer strands or rods are often much less regularly arranged, and may appear to cross one another diagonally, or still to be connected by cross-threads (I, 3; VI, 5, 7). I have very few examples of this stage in eggs which belong at all certainly to the second ("spherical nucleus") class, and in them the arrangement of the inner group of strands is so much less regular that it suggests a rather different process (VIII, 2; VII, 7), which will be discussed after the eggs with "top-shaped" nuclei have been considered.

In the eggs of the first class ("top-shaped" nuclei), when the inner group of strands has become arranged as a sheaf of rod-shaped chromosomes on a spindle, the outer group may either follow suit and form a similar group of rods, usually, however, less regular (II, 4; III, 5, 6), or, probably more commonly, a division of the inner group takes place before the outer group has formed any very regular arrangement (V, 1, 2), but in these eggs the division of the outer group takes place quite definitely, though it may lag somewhat behind that of the inner group (IV, 5; I, 4). Although it is quite clear that a double division takes place, I am quite unable to describe in detail how it is effected, for I have failed to find any eggs (except, perhaps, that shown in Fig. X, 2) in stages between that represented in Figs. II, 4, and III, 5, and that shown in I, 4, IV, 5, and VI, 1, 2.

In the earlier stages the chromosomes consist of fairly thick rods (presumably ten, though usually aggregated so as to appear fewer), lying lengthwise on a spindle. In the next stage represented these chromosomes have divided into a group of about ten rods, which sink in to form the egg-nucleus, and ten others, which remain as the inner group of polar chromosomes, but the figures give no indication of whether the division is longitudinal or transverse. The arrangement of the chromosomes on the spindle would suggest a transverse division; the existence of ten rather long, narrow rods in both the egg-nucleus and inner polar group suggests that the division is longitudinal, and this is confirmed by the longitudinal split which is sometimes visible (Fig. X, 1), corresponding with the apparently double

nature of the original strands from which the chromosomes were formed. Without deciding definitely, therefore, it may probably be concluded that the division is longitudinal, and that each of the rods arranged lengthwise on the spindle splits longitudinally, and one half slips over the other and passes to the inner pole, leaving the other nearly stationary. The form of some of the chromosomes represented in Fig. X, 2, might correspond with a division of this sort. That such a division is very unlike what is found in other organisms is obvious, but it appears to be most nearly what the figures observed suggest.

The division of the outer group which follows is perhaps of the same kind, as is indicated by such figures as X, 5, in which some of the separating chromosomes appear to be attached to one another in such a way that the outer end of the inner chromosome is lying parallel and in contact with the inner end of the outer. In other cases the figures give the appearance of a sorting out of the chromosomes of the inner group, rather than of a division (I, 4; X, 4), but the fact that after the two groups have separated some chromosomes may remain connected by long chromatin strands\* rather indicates a genuine division. The final result of the division of the outer group of chromosomes in eggs of this class is usually a rather regular group of about 10 chromosomes arranged in a sheaf near the edge of the egg, and a less regular group at a somewhat deeper level (Figs. IV, 5, 6; III, 7).

The above description applies to eggs of females whose eggs in their earlier stages have "top-shaped" nuclei, and my few good figures of eggs of the "spherical nucleus" class make it doubtful whether the divisions are quite similar. The only really satisfactory figure of the stage immediately preceding the division of the inner group of chromosomes is shown in Fig. VIII, 2, and it will be seen that in this case the inner group, instead of consisting of a regular sheaf of rod-like chromosomes (*cf.* Figs. II, 4; III, 5) is made up of a rather irregular assemblage of rods and strands. It is possible that this appearance is due to the chromosomes being actually in division, and in process of separation from one another, for the number is clearly more than 10, though the irregular arrangement makes an exact count impossible. A rather later stage is shown in VII, 7, in which eight or nine chromosomes are clearly seen sinking in to form the egg-nucleus, while those of the outer half of the inner group have loop-like connections with one another. In both figures the chromosomes of the outer group show no clear sign of division at this stage, though traces of spindle fibres may be seen running outwards towards the edge of the egg. Fig. VII, 8, *a, b*,

\* *Cf.* Part I, Plate 2, fig. 32, 'Roy. Soc. Proc.' B, vol. 82.

shows the polar chromosomes of an egg of this series after the maturation divisions are completed; there is a compact mass derived from the outer half of the inner group (on the right of VII, 8, *a*, with three pieces extending into the section 8, *b*), while the halves of the outer group are intermingled in an irregular mass extending through the two sections. As in the case of the earlier stages, however, various intermediate conditions occur between such figures as VII, 8, and III, 7, and it is possible that the two kinds of eggs described are rather the extremes of a continuous series than sharply distinct types which might be correlated with a difference in sex-production in the flies to which they give rise.

One other point should be noted. In several figures (*e.g.*, VI, 7; VII, 7) there is a more or less conspicuous lagging chromosome, which is possibly derived from one of the chromatin strands which may connect the inner and outer groups after the first division (Figs. X, 1, 2; VI, 5). I have not been able to find any regularity in its occurrence, but if one chromosome differs from the rest in tending to lag in division, the presence of this chromosome in the inner or outer group might possibly be the differentiating factor which determines whether the egg becomes a male-producing or female-producing individual.

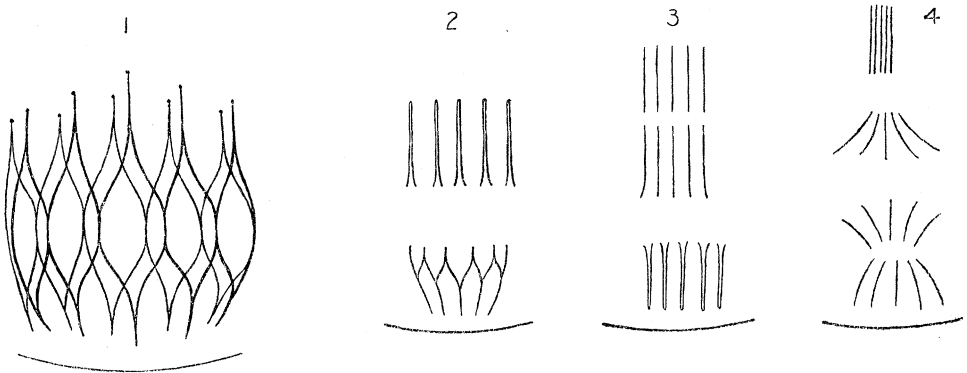
The type of mitotic figure described, which occurs in the maturation division of both the sexual and parthenogenetic eggs of *Neuroterus*, and which is very similar to that described by Henking\* in another Cynipid, *Rhodites*, is so different from that commonly found in nearly all other organisms that some further discussion of its peculiarities seems needed. An obvious suggestion is that it is due to defective preservation, and this explanation is supported by the fact that the state of preservation of the eggs seems to differ considerably in eggs fixed together in the same tube. But on the other hand there are strong reasons for believing that this is not the true, or at least not a complete, explanation.

In the first place quite similar figures are found in eggs fixed with Petrunkevitch's fluid and Gilson's acetic alcohol sublimate, both of which, however, are of similar constitution, but are very different from the fixatives (hot water and Flemming's fluid) employed by Henking. More important reasons for believing that the peculiarities observed are not due to bad fixation are (1) that quite normal and well-preserved segmentation mitoses occur in rather older eggs, and (2), the beautiful clearness and regularity of the fine chromatin strands could hardly be produced by poor fixation. The drawings give only an imperfect representation of the fineness and regularity of the actual figures, owing to the difficulty of rendering them accurately in

\* 'Zeit. Wiss. Zool.,' vol. 54, p. 147 (1892).

perspective. Further, although there are considerable differences in detail, in the rather earlier stages especially there is a uniformity about the figures which makes it difficult to believe that the appearances are due to artifact.

I am inclined, therefore, to believe that the figures are at least to a considerable extent genuine representations of what exists in the living egg, and that the maturation divisions of the Cynipidae exhibit a type of mitosis not hitherto observed in other forms. Its essence appears to be the drawing out of threads from a reticulum; the threads, at least at their bases, are double, and the ends of the longitudinal halves diverge and anastomose with the threads of the network. Gradually the network disappears, apparently becoming absorbed into the drawn out threads, forming an inner and outer group. These two groups then each divide, but, as was said above, there are no clear indications of how this second division is effected; it is probable, however, that the division consists in a separation of the longitudinal halves of the originally double threads. To make this description more intelligible, I give in a text-figure a purely diagrammatic representation of the kind of



DIAGRAMS 1-4.—Representing the kind of mitotic division suggested by the figures observed in the eggs of *Neuroterus*.

1. Threads becoming drawn out on opposite sides of a reticular nucleus.
2. Separation and sinking-in of the inner threads, each of which is longitudinally split.
3. Division (longitudinal ?) of the inner threads, and formation of split chromosomes from the outer group.
4. Completion of the maturation divisions. The innermost group forms the egg-nucleus, the other three the groups of polar chromosomes.

(In diagrams 2, 3, 4, only half of the actual number of chromosomes is represented).

system which the figures suggest, but it will be understood that this representation is imaginary, and is not intended to show at all accurately the structures as they actually exist.

*Summary.*

In *Neuroterus lenticularis* there are two generations in the year, agamic females appearing in early spring, and sexual females and males in early summer. Previous work had shown that any individual agamic female has only male or only female offspring, and the object of the present work was to discover the nature of the difference between these two classes of agamic females.

Experiment showed that any individual sexual female has grandchildren exclusively or almost exclusively of one sex. The galls produced by sexual females were sleeved, each sleeve containing galls derived from one female parent, and in sleeves of galls derived from six females 4235 males and 83 females were bred, while in sleeves of galls from another six females there were 5139 females and 117 males. About half the sleeves contained no exceptions, and reasons are given for believing that the exceptions were due to eggs of wild flies laid through the meshes of the sleeves.

Two possible cytological causes might account for the fact that some sexual females produce only male-producing offspring, whilst others produce only female-producing. If each fly pairs only once, the difference might depend on the existence of two kinds of males, or it might arise through differences in the maturation-processes of eggs laid by the two classes of sexual female. No cytological differences in the spermatogenesis of different males could be detected. The maturation phenomena of the eggs (about 300) of 15 separate females have been examined, and while they seem to fall into two rather distinct types, the differences are not sufficiently considerable to correlate them with the sex-phenomena with any confidence.

The maturation-processes of the eggs are remarkable, and if, as seems probable, the peculiar figures are not due to methods of preservation, they differ widely from the ordinary type of mitosis. The first division takes place by the drawing out of threads (probably double) on each side of the nucleus; the reticulum becomes absorbed in these threads, which form two groups of parallel chromosomes on a spindle. These chromosomes then divide, probably longitudinally, giving rise to the group which forms the egg-nucleus and three groups of polar chromosomes.

[*Postscript, February 19, 1916.*—While the preceding paper was in the press I received R. W. Hegner's paper "Protoplasmic Differentiation in the Oocytes of Certain Hymenoptera."\* He describes phenomena in the parasitic Hymenopteran *Copidosoma*, and less fully in the Cynipid *Andricus* (the latter

\* 'Journ. of Morphology,' vol. 26, p. 495 (1915).

nearly allied to *Neuroterus*), which may throw important light on the nature of the peculiar mitotic figures found in the maturation divisions of *Neuroterus* eggs. In the early oocyte, after a compact synizesis (contraction phase) a number of thread-like chromosomes emerge from the spireme, appear to pair end to end, and the pairs then arrange themselves as parallel rods on an asterless mitotic figure. Each of these double chromosomes shows its double nature by a transverse projection in the middle, so that it has the form of a cross with the horizontal arms very short. The whole group of chromosomes then condenses till it makes an evenly stained mass which is more or less oval in shape. This is the last stage seen before the egg is laid, and evidently corresponds with the small evenly-stained nucleus at the edge of the egg found in eggs of *Neuroterus* preserved immediately after deposition. It is extremely like Figs. 1 and 2 of Plate 17 in Part II of this series.\*

Further, F. Martin† finds the same phenomena in *Ageniaspis*, followed by a maturation division apparently of the type described in *Neuroterus*.

It seems very probable, therefore, that the "top-shaped" nucleus described in *Neuroterus* is a stage in the disentangling of these chromosomes from the compact condition, and that the separation of the inner group from the outer in the first division corresponds with a separation of the members of the end-to-end pairs from one another, while the second division would be, as suggested above, a longitudinal splitting of single chromosomes. The peculiarities of the first division may be supposed to arise from the fact that the double chromosomes prepare for division some time before the egg is laid, then become closely pressed together into a compact mass, and after the egg is laid separate from one another before they are completely disentangled from the tight mass into which they have been concentrated.]

\* 'Roy. Soc. Proc.,' B, vol. 83 (1911).

† 'Zeit. Wiss. Zool.,' vol. 110, p. 419 (1914).

## DESCRIPTION OF FIGURES.

The figures are all freehand drawings made with a Zeiss 2.5 mm. immersion lens (N.A. 1.40), and compensating ocular 12. They are not all exactly on the same scale. In many of the figures, especially those representing the earlier stages of the maturation divisions (*e.g.*, Series IX), the difficulty of interpreting and representing the structures in perspective with complete accuracy has proved insuperable, and the figures must therefore be taken as giving as close a rendering as I am able to do of the appearance presented, rather than as drawings which are accurate in every detail. In each case the line on the left of the figure represents the position of the egg-margin. The Roman numerals represent (except in Nos. IX and X) different individual females, so that, for example, Figs. VII, 1-8, represent eight maturation figures from eggs laid by one parent. Series IX and X represent figures at various stages of division from eggs of mixed females; of these, Figs. IX, 1-6, and X, 3 and 5, were preserved in 1907; X, 1, in 1906; X, 4, in 1914, and X, 2, in 1915. All the eggs of Series I-VIII were preserved in 1914 or 1915.

SERIES I, 1, 2.—“Top-shaped” type of nucleus just before maturation. In I, 2, the stained granules, suggesting emission of chromatin, are seen round the equator.

I, 3.—Early stage of division; since in some cases the chromosomes are lying one directly over another not all are shown.

I, 4.—Later stage of division. The inner group has divided into egg-nucleus and inner polar group; the outer group has not yet divided. The figure is reconstructed from four successive sections.

SERIES II, 1 and 2.—Top-shaped nuclei with double rings of granules.

II, 3.—Prophase with narrow loops or strands becoming drawn out on the inner side.

II, 4.—Unusually regular division-figure. The inner and outer groups are in successive sections, with the exception of the chromosome marked  $\times$  in the outer group, which is in the same section as the inner group. On the inner side of the inner group 10 spindle-fibres can be counted with some confidence.

II, 5.—Three groups of polar chromosomes after the completion of the maturation divisions. The lowest and that near the edge are the halves of the outer group and are in one section; the upper on the right hand is the inner group containing 10 chromosomes, and is in the next section.

SERIES III, 1, 2, 3.—Prophases; III, 1, has the typical top-shaped form with equatorial granules.

III, 4.—Chromatin strands being drawn out on both inner and outer sides. Owing to superposition and perspective, not quite everything is shown.

III, 5, 6.—Two double division figures, on rather a larger scale than the remainder. In each case the outer group is in one section, the inner in the next.

III, 7.—Completion of maturation division and sperm-head (*Sp.*) swelling up to form male pronucleus. The two outer groups of polar chromosomes (the outermost with about 10) and the inner group are in one section, with the exception of the chromosomes represented separately below, which are in the next. The egg- and sperm-nuclei (marked  $\varphi$  and *Sp.*) are both in the next section but one.

SERIES IV, 1 and 2.—Early stages seen in face, cut in two sections. In each the upper figure represents the inner, the lower the outer portion. In IV, 2, there are clearly about 10 threads drawn out towards the egg-centre; in the lower figure the dark spots are the cut ends which are continuous with the threads in the upper figure.

IV, 3.—Inner portion of a similar figure, showing that the threads, at least near their bases, are double.

(IV, 1, 2, 3, should be compared with VII, 2, 3, 4, 5.)

IV, 4.—Two figures from successive sections of a rather later stage in side view, showing chromatin threads drawn out on both inner and outer side. The staining of this slide (IV, 4) is unsatisfactory, and attempts to improve it have proved unsuccessful. The figures are therefore to be taken as sketches.

IV, 5.—Telophase of maturation division. The egg- and sperm-nuclei are in one section, but are represented nearer together than they actually are; the two inner polar groups and most of the outer are in the next section but one. Some of the outermost polar chromosomes are hidden by the egg-membrane.

IV, 6.—Polar chromosomes after the completion of the maturation divisions from three successive sections. Those of the inner group are clumped.

SERIES V, 1 and 2.—Two rather similar stages, showing complete separation of egg-nucleus chromosomes from inner polar group before the outer group has divided. In V, 1, the figure is reconstructed from two, in V, 2, from three, successive sections, and the chromosomes are seen nearly in face. The later eggs of this series show three groups of polar chromosomes, indicating that the outer group divides.

SERIES VI, 1, 2, 3.—Three prophases somewhat intermediate between the "top-shaped" and "spherical" types.

VI, 4.—Later stage showing threads, double at their bases, drawn out towards the egg-centre and shorter loops towards the outside. The lower figure represents ends of threads seen in the next section; the upper figure is slightly spread for the sake of perspective.

VI, 5, 6.—Rather later stages: VI, 5, is cut in two sections; VI, 6, is probably not well preserved, the inner and outer groups are in successive sections.

VI, 7.—Double division-spindle, with a lagging chromosome in the outer group.

SERIES VII, 1.—Spherical nucleus before maturation.

VII, 2.—Two sections of a later stage in face. The upper figure represents the inner, the lower the outer, portion; in the latter, the dark spots are the cut ends of threads which appear in the upper figure.

VII, 3.—Oblique view of slightly later stage, showing double threads drawn inwards towards egg-centre.

VII, 4.—Similar stage cut in two sections in face. The upper figure is the inner, and the dark dots in the lower are the cut ends of threads which pass into the upper.

VII, 5.—Similar figure, showing double nature of the threads towards their bases. The 10 double threads forming an irregular outer ring are probably chromosomes; the three smaller pairs inside the ring are much fainter, and are possibly spindle fibres.

VII, 6.—Oblique view of similar stage.

VII, 7.—Mitosis in side view, showing complete separation of the egg-nucleus chromosomes from the inner polar group, while the outer group shows

little trace of division. The innermost group is in one section, the middle group and most of the outer group in the next. The chromosomes of the outermost group not in this section are represented below.

VII, 8.—Polar chromosomes after maturation is completed, in two successive sections. There is a compact inner group of about 10, and an outer group which contains approximately 20 chromosomes.

SERIES VIII, 1.—“Spherical” type of nucleus with early stage of threads being drawn out towards egg-centre.

VIII, 2.—Double maturation division. There appear to be more than 10 chromosomes in the inner group, and they seem to be in process of sorting out rather than of division. The inner group is in one section; the chromosomes of the outer group marked  $\times$  in the next, and the rest of the outer group in the next to these.

SERIES IX, 1-6.—Six figures of early stages from eggs of various females, preserved with Petrunkevitch's solution in 1907, and showing strands or loops of chromatin being drawn out towards both the egg-centre and egg-margin from a nuclear reticulum. In IX, 6, the figure is seen obliquely, and is cut in two sections; the upper portion is the outer.

Owing to the difficulty of interpreting and rendering structures seen in perspective, the details of the reticulum as represented are not rigidly accurate, nor is the whole thickness of the nucleus shown in every case.

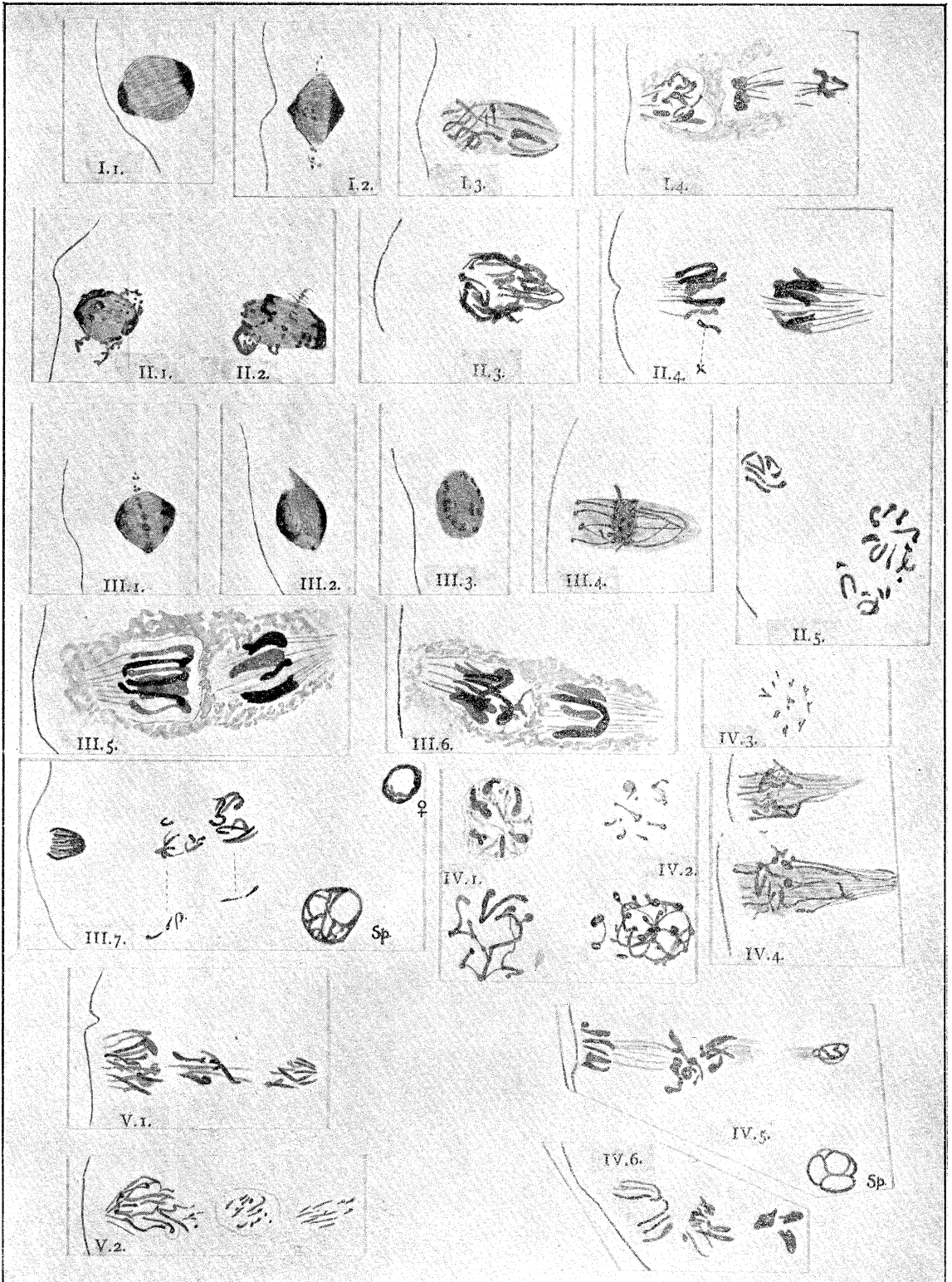
SERIES X.—Five figures of eggs of various females, later than those shown in Series IX.

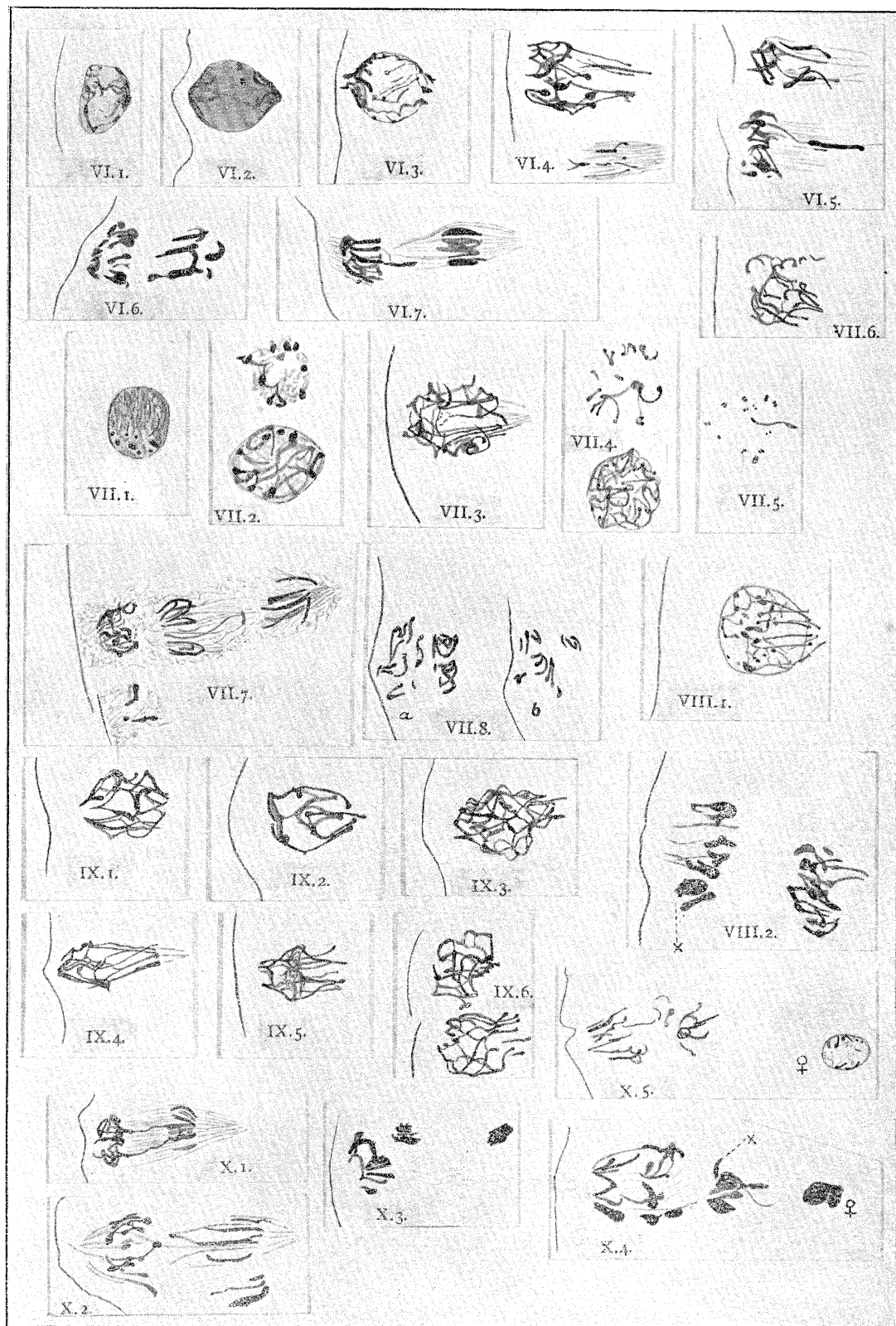
X, 1.—Egg preserved in 1906 (Petrunkevitch's fluid), showing chromatin strands connecting the inner and outer groups. Eight chromosomes, most of them clearly split longitudinally, are visible in the inner group; it is possible that the two long strands represent lagging chromosomes.

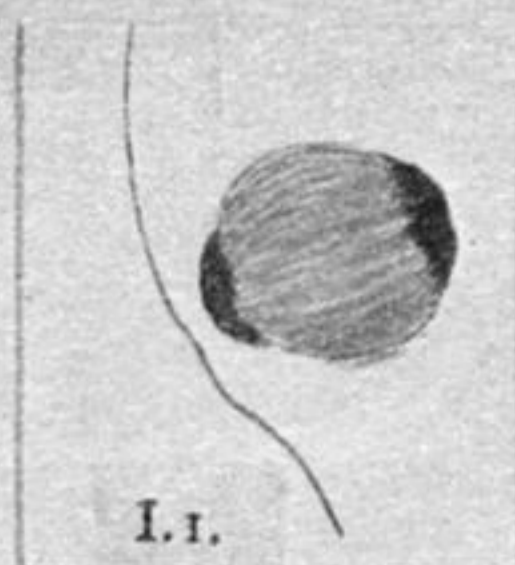
X, 2.—Egg preserved in 1915 (Gilson's acetic alcohol sublimate). The chromosomes below those of the inner group on the spindle are in the next section to the remainder. The thick, deeply stained chromosomes of the inner group are probably each two in contact; it is not certain whether the thin faint lines between them are single chromosomes or spindle-fibres.

X, 3-5.—Three stages after the division of the inner group is complete, showing division of the outer group. X, 3 and 5, preserved in 1907; X, 4, in 1914. In X, 4, the outer group and all the chromosomes of the inner, except that marked  $\times$ , are in one section; the chromosomes of the egg-nucleus (marked  $\varphi$ ) are two sections removed.

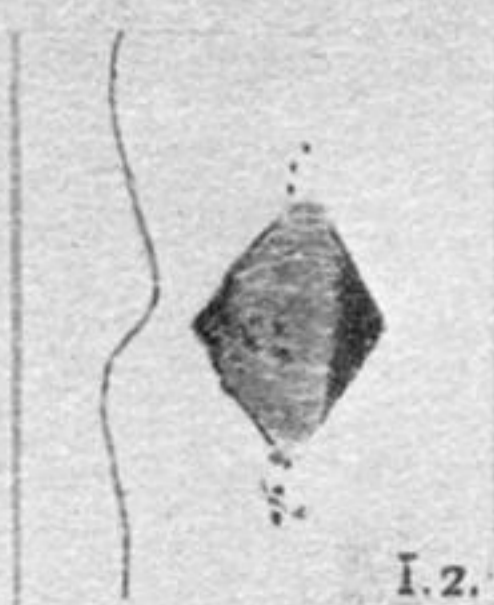
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I.2.



I.3.



I.4.



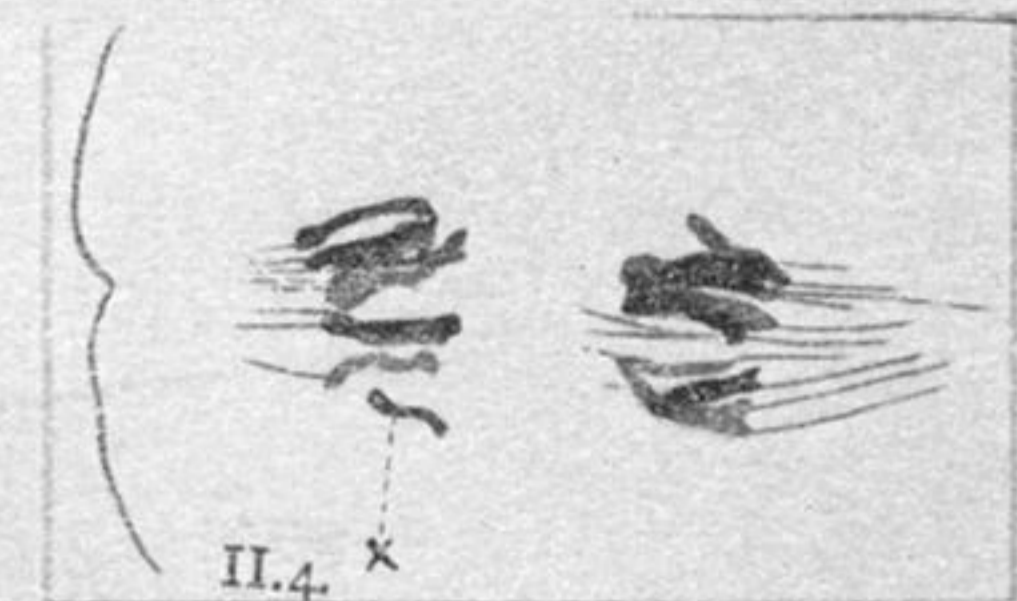
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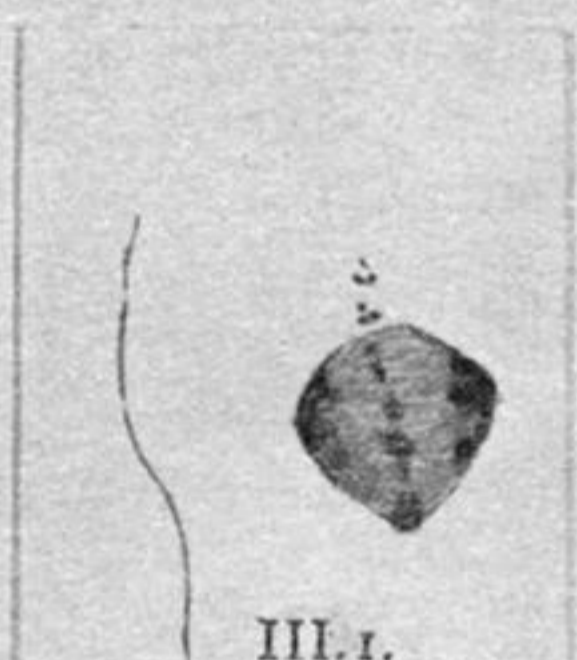
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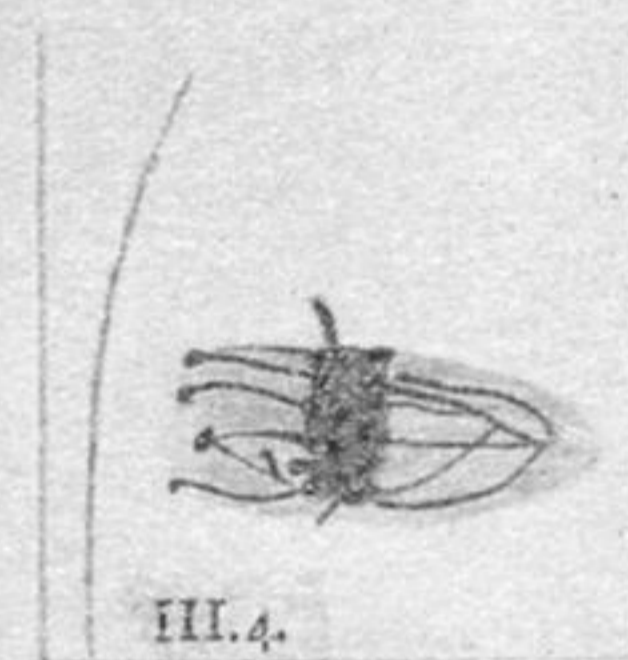
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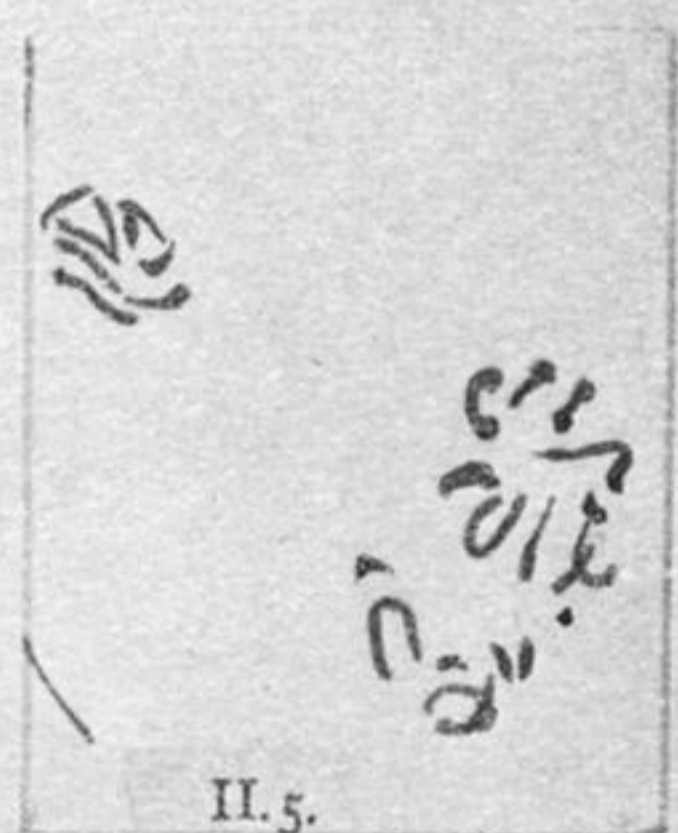
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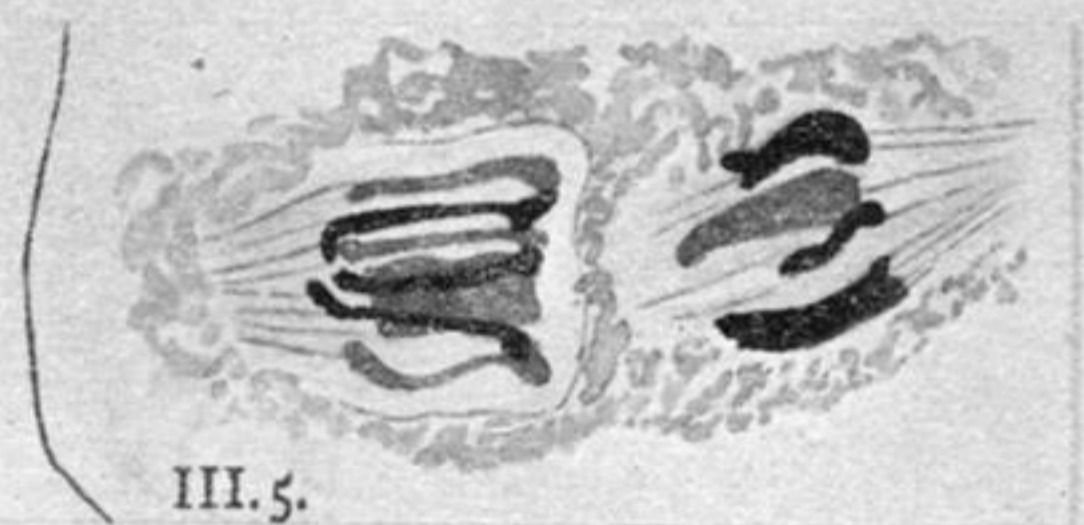
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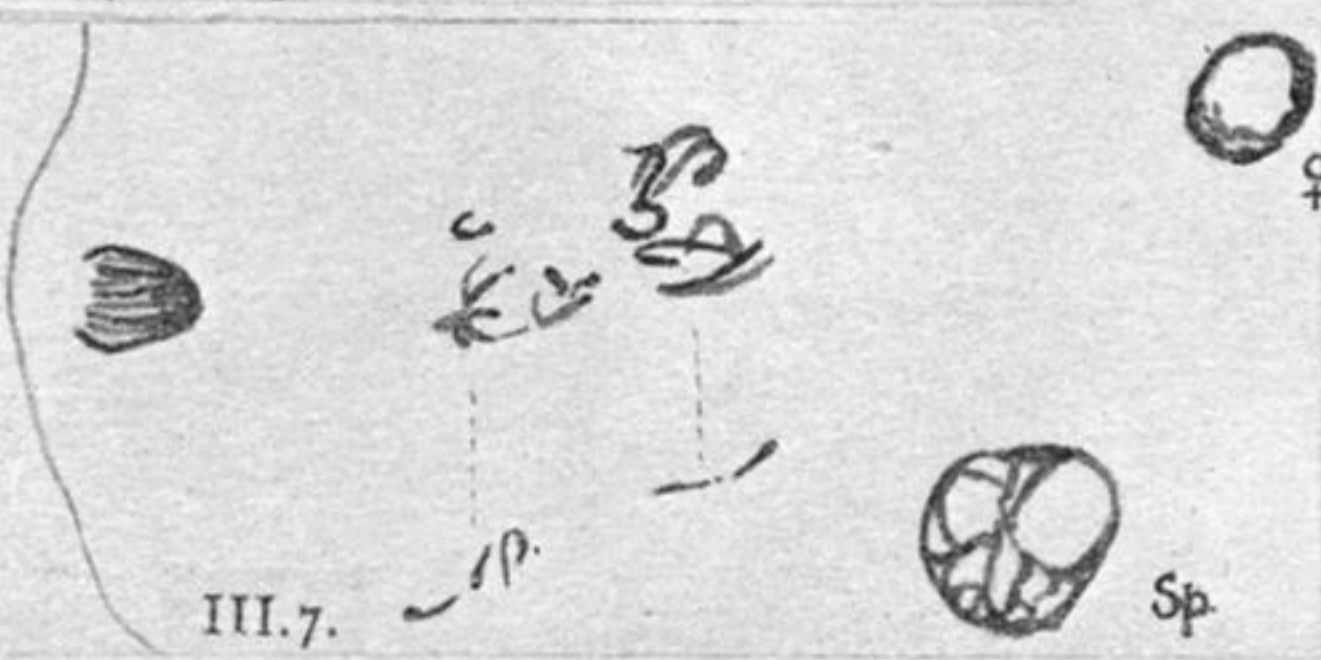
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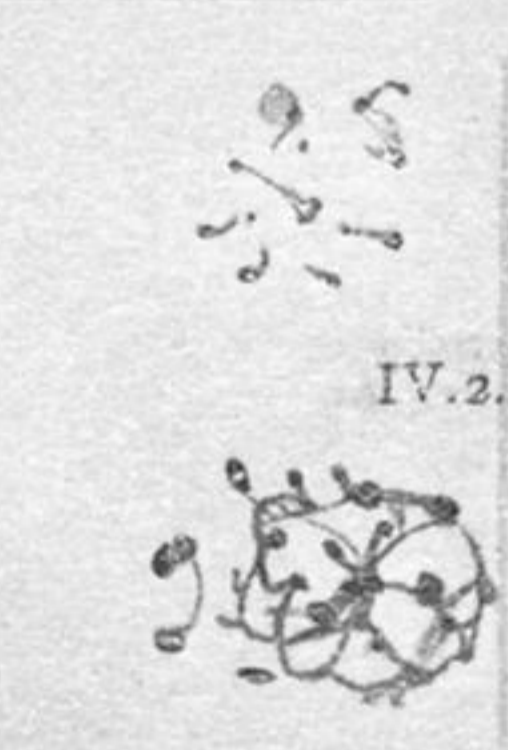
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III.7.



IV.1.



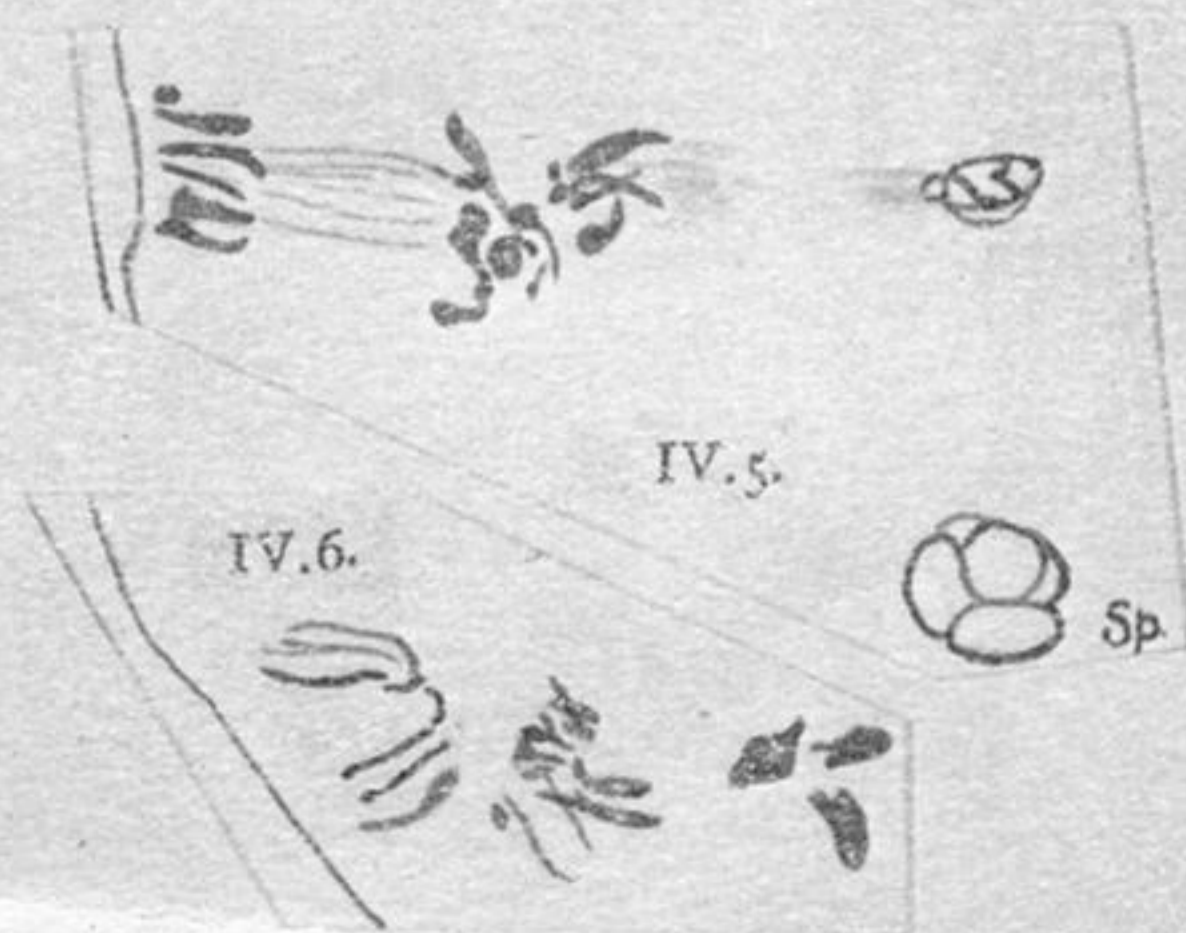
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IV.4.



V.1.

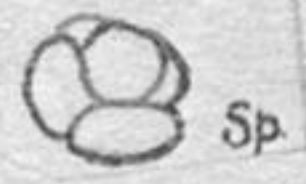


IV.5.



V.2.

IV.6.



Sp.



VI.1.



VI.2.



VI.3.



VI.4.



VI.5.



VI.6.



VI.7.



VII.6.



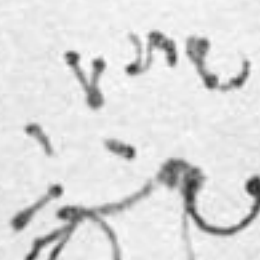
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VII.2.



VII.3.



VII.4.



VII.5.



VII.7.



VII.8.



VIII.1.



IX.1.



IX.2.



IX.3.



VIII.2.



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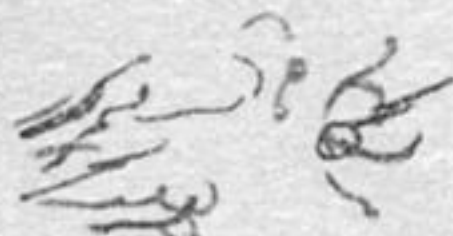
IX.4.



IX.5.



IX.6.



X.5.



X.1.



X.3.



X.4.



X.2.