

SEXUALITY OF FILAMENT OF SPIROGYRA¹

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(WITH PLATES XXIII-XXV)

Is the filament of *Spirogyra* unisexual or bisexual? This has been a question for many years, but the reports of the great majority of modern workers would indicate that they regard the filament as wholly of one sex. The answer to the question hinges upon the presence of zygotes in both of the conjugating filaments. If they occur in only one of the two, the filaments may be said to be unisexual, since one functions as the male and the other functions as the female; if, on the other hand, they occur in both filaments and are formed by scalariform conjugation, the filaments may be said to be bisexual, since the empty cells in each have furnished the male gametes, while the ones in which the zygotes occur previously contained female gametes. The latter case is known as cross-conjugation.

The advocates of the theory of the unisexuality of the filament urge that as a rule all the male gametes arise in one filament and pass over into the other, so that zygotes appear in but one of the two conjugating filaments. They urge also that cases of cross-conjugation are so rare that they should be considered abnormalities and the result of forced conditions. Strangely enough they ignore lateral conjugation in so far as the sexuality of the filament is concerned. On the other hand, those who do not accept this theory have taken two different positions: one, that the gamete is absolutely sexless (HASSALL 17, p. 130; also PRINGSHEIM, see BENNETT 2); and the other, that the filament is bisexual, based

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principally upon the fact of lateral conjugation. Occasionally a case of cross-conjugation is presented as evidence, but these cases have been so isolated that they have been of little value. A clear case, then, of cross-conjugation occurring normally in a species would certainly strengthen the position of the advocates of the bisexuality of the filament.

VAUCHER (28), who was apparently the first to work upon *Spirogyra*, at least upon the problem of its reproduction, figures it in most cases in cross-conjugation. Figs. 1-4 of pl. XXIII are photographic copies from his plates. In his general description of the *Conjuguee* he says "ordinarily one of the filaments gives while the other receives throughout its entire length. Still it is not rare for the same filament to give in one part of its length and receive in another in such a manner that some of the cells of the tube are filled and some are emptied." Again, "while a tube usually gives or receives throughout its entire length, still it frequently happens, as I have said before, that some give and receive almost alternately." In regard to *Conjuguee condensée*, he says "the berries are indifferently lodged in either tube" (VAUCHER 28, p. 69).² Such statements seem to point clearly to cross-conjugation, but when we remember that VAUCHER, probably because he was unacquainted with the phenomenon, did not figure or describe lateral conjugation, and that he was dealing in part with species which normally reproduce in this manner, we have reason to believe that he was really observing a combination of lateral and scalariform conjugation which might easily have been mistaken for cross-conjugation. Just such conditions have been observed by the writer. Pl. XXIII, fig. 9, shows diagrammatically such a case that came under his observation. At first glance the position of the zygotes in the 2 filaments gives the appearance of cross-conjugation. Upon closer investigation it is found that the contents of cell (a) probably passed into (b), (c) and (d) represent another lateral conjugation, (e) and (f) have conjugated in the regular scalariform manner with (g) and (h), (i) and (j) are lateral, and (k) entirely failed to conjugate.

² Free translations.

VAUCHER has figured 6 species of *Spirogyra*, 2 of which are shown in scalariform conjugation and 4 in cross-conjugation. I have arranged these species in table I so that the methods of reproduction assigned by the different writers may be compared.

TABLE I

SPECIES ACCORDING TO VAUCHER	METHOD OF REPRODUCTION		
	Vaucher	Wolle	DeToni
Allongee.....	Scalariform	Scalariform	Lateral
Adherente.....	Scalariform	Scalariform	Not stated
Majeure.....	Cross	Scalariform	Not stated
Portiquis.....	Cross	Scalariform and aplanospores	Not stated
Condensee.....	Cross	Scalariform	Often lateral
Renflee.....	Cross	Scalariform and lateral	Often lateral

At first glance there does not seem to be much uniformity, but if we assume that where DETONI does not state the particular method of conjugation that the scalariform method is meant, we find that WOLLE and DETONI agree that 5 of these species have scalariform conjugation. They also agree in assigning lateral conjugation to *renflee*. However, DETONI alone states that *condensee* conjugates laterally, while WOLLE alone states that *portiquis* forms aplanospores. In these 3 cases, therefore, it would be possible to have combinations with the appearance of cross-conjugation. *Majeure*, however, according to both WOLLE and DETONI, conjugates only in the usual scalariform manner. Figuring this species in cross-conjugation by VAUCHER is rather surprising, as will appear in the discussion which follows.

Since there have been but few cases of cross-conjugation reported, we have reason to doubt its occurrence in as many species as figured by VAUCHER. Furthermore, no other observer up to the present time has recorded its occurrence in any of these species. However, the species discussed in the present paper has been tentatively identified as *S. inflata* (Vauch.) Rahb., which according to RAHBENHORST would include *Conjuguee renflee* of VAUCHER.

Recognizing, then, the facts that (1) VAUCHER was unacquainted with lateral conjugation, (2) a combination of lateral

and scalariform conjugation resembles true cross-conjugation in appearance, (3) he has figured cross-conjugation in 3 species in which this appearance would naturally occur, due to this combination, (4) no other observer has reported any of these species in cross-conjugation, the writer feels that there is good ground to doubt the observation of true cross-conjugation by VAUCHER.

HASSALL (17) also has figured cross-conjugation, but does not describe it for any of the species thus figured, although he does mention it in the general description of the Zygnemaceae. Figs. 6 and 8 of pl. XXIII are reproductions from his plates showing this phenomenon. While HASSALL figures and describes cross-conjugation, he nowhere claims to have observed its occurrence. One of the contemporaries of HASSALL, in reviewing his book (HASSALL 18), says "it is unfortunate that the author has not pointed out the cases in which the figures are not the result of his own observations but copied from published plates." Certain cases are cited in which HASSALL's plates were taken from published plates, and these tend to cast some doubt on the source of his plates on cross-conjugation, although they are not among those cited. The writer has been seeking the originals of these borrowed plates, but as yet has not been able to locate them, and is therefore uncertain as to whether or not they exist. BENNETT (2, p. 432), in reviewing the general field, says in regard to HASSALL "it is quite possible that the statement may be the result of an error of observation; I have often been deceived in this way." Further, HASSALL claims to have discovered lateral conjugation, and with this as his basis he lays great stress upon the act of conjugation as being without sex, explaining the movement of the gamete by the "law of universal gravitation" (HASSALL 17, p. 132). He gives the following reasons for his belief: (1) both cells are alike; (2) reproductive bodies are surrounded by the heavy wall solely for protection; (3) spores arise in the same species, both with and without conjugation; and (4) there is conjugation but "no mixing of the endochrome." As a conclusion he says "thus, so far as can be presumed, the information already acquired would be opposed to the belief in the existence of sex as applied to the cells of *Conferva*."³

³ This genus as used by HASSALL included *Spirogyra*.

With this as a basis one is not surprised to find him ascribing cross-conjugation as a character of the genus.

BENNETT (2) states that CLEVE (8) has figured 2 cases which may be called cross-conjugation. The writer, however, thus far has been unable to secure this monograph.

BESSEY (4) figures a case which he calls cross-conjugation. This figure is reproduced here (pl. XXIII, fig. 7). It is to be noted that in his description no mention is made of cross-conjugation, although it is made later (BESSEY 5). If the figure is complete, it is by no means a conclusive case, as it shows only one pair of conjugating cells. The presence of a zygote in filament *A* can be explained as the formation of an aplanospore after the cell had put forth the conjugating tube. WEST (29, fig. 64) shows a case of false cross-conjugation occurring in this manner. The idea is further supported by the observation of the writer, and diagrammed on pl. XXIII, fig. 9. Again, the cell in the filament *A*, if in cross with *B*, should bear an oval zygote, since the oval form seems to be dominant to the spherical. This fact was observed by BESSEY, as he states that the zygote of *S. protecta* is oval in shape, but those of *S. majuscula* are spherical, and that the hybrid between these two assumes the oval shape characteristic of *S. protecta*. He does not apply this, however, to the zygote formed in filament *A*, which is spherical. If the cell referred to is in cross with another filament, that filament should be shown. The figuring of the canal is not sufficient evidence, as has been shown. Furthermore, this hybrid is undoubtedly a forced condition. The strenuous efforts of *S. majuscula* to reproduce are shown in another case cited by BESSEY (6), in which this species tries to hybridize with *Mesocarpus*.

BENNETT and MURRAY (3, p. 266) say "as DEBARY (11) has pointed out, . . . one of the two filaments is entirely emptied, while the other is completely filled with zygospores." To this they have added a footnote "HASSALL, however, figures and asserts to the contrary."

WEST (30, p. 125) refers to the footnote of BENNETT and MURRAY, but maintains that the phenomenon is rare. He states that he has seen but a single case, and that was in *S. gracilis* (WEST 29, p. 47). G. S. WEST, in a more recent personal com-

munication, says that he regards the phenomenon as rare, not having seen more than half a dozen cases of it. However, he sees no reason why it should not occur, as it represents much the same phenomenon from the sex standpoint as lateral conjugation. In both cases there must be a differentiation of sex in the one filament. A very distinct difference, however, will be pointed out later.

COULTER (10, p. 40) briefly describes cross-conjugation but does not assign it to any species.

On the other hand, the great majority of botanists doubt the occurrence of cross-conjugation, and with it the bisexuality of the *Spirogyra* filament. AGARDH (1), according to HASSALL (15), states that one filament is always giving and the other always receiving. WOOD (32) mentions scalariform and lateral conjugation, but not cross-conjugation. COOKE (9) has 11 plates of *Spirogyra*, but does not figure cross-conjugation. DEBARY (11) states that one filament gives and the other receives. WOLLE (31) figures and describes 39 species of *Spirogyra*, citing VAUCHER and HASSALL, but does not mention cross-conjugation. HABERLANDT (14), according to KLEBS, holds that the filaments are distinctly sexual. In order to verify this statement, KLEBS (22) grew *Spirogyra* on nutrient agar, but found that a filament would not conjugate with itself, and therefore concluded that it was all of one sex. This experiment might prove the case for one species, but it hardly appears just to use it as the basis for a sweeping statement that bisexuality does not occur in *Spirogyra*. MOTTIER (24) cites this experiment as a basis for belief in the unisexuality of the filament. DETONI (12) absolutely ignores cross-conjugation, although he cites the plates of both VAUCHER and HASSALL in his descriptions of the species figured in this condition by them. LOTSY (23) states that there is a distinct difference between the male and the female filaments. OLTMANNS (25, p. 64) states specifically that we have to do with male and female filaments. HERTWIG (19) makes a similar statement. ENGLER and PRANTL (13) speak of the visible difference between the male and female filaments. ROBERTSON (26), who grew *Spirogyra* extensively under abnormal conditions, did not find a case either of cross or lateral conjugation. Since cross-conjugation did not occur in his

own experiments and is so exceedingly rare in the work of others, he thinks it must be considered a very unusual abnormality. YORK (33), who worked several years on the sexuality of *Spirogyra*, seeking methods for determining the sex before conjugation, says "zygotes were never found in both filaments, but only in the one containing the greater amount of food. The male and female are morphologically and physiologically different."

Summarizing, it appears that the evidence for bisexuality is based (1) upon work done over 100 years ago, when the importance of cross-conjugation was not realized, and has not been verified since; (2) upon lateral conjugation, a strong basis ignored by the unisexualists; (3) upon the chance observations of CLEVE, BESSEY, and WEST. At this point it is interesting to note that HASSALL figures no species in cross-conjugation that was thus figured by VAUCHER; that BESSEY's species is not that of either HASSALL or VAUCHER; and that the one cited by WEST is still different. Thus these would all appear to be abnormalities.

On the other hand, the advocates of unisexuality urge (1) that KLEBS found that a filament would not conjugate with itself, hence it is of one sex; (2) that the work of VAUCHER needs verification; (3) that the figures of HASSALL may have been taken from older works; (4) that, since the species figured by VAUCHER and HASSALL are common, the phenomenon should have been observed by modern investigators; (5) that specialists have seen but few cases, not more than a dozen, and these have been called abnormalities because of their rareness; (6) that experimentalists who have spent years on the sexuality and abnormal conjugation of *Spirogyra* have not observed cross-conjugation. All these things point to the unisexuality of the filament.

If, however, as stated in the beginning of this paper, a true case of cross-conjugation of *Spirogyra* should occur normally to any extent, it would settle the question, for one species at least. A species in this condition was found by the author while making a collection of algae along a stream near Durham, North Carolina, on April 1, 1915. The water stood in pools on the low ground, and it was from one of these pools that the collection was made. There was comparatively little of this species mixed with a larger *Spiro-*

gyra and some germinating *Vaucheria*. The phenomenon of cross-conjugation was not observed until the material had been brought into the laboratory, and heavy freshets prevented further collection. However, from this interwoven mass, not larger than a pea, more than 70 slides have been prepared showing cross-conjugation. Some of the slides have several distinct pairs of filaments in this condition. Considerable effort was made to secure long filaments, but this was unsuccessful on account of the intricate tangling of the mass. This species was again collected early in April 1916, in approximately the same locality, showing essentially the same phenomena as the earlier collection, but, owing probably to deficient rainfall, was not abundant and hence it was not possible to add any important facts not shown by the material gathered the year before.

A careful investigation of the material shows that all the known forms of reproduction in *Spirogyra* are represented in this species. While aplanospores occur, they are not found frequently, and they are hard to identify. The regular zygotes are formed by 3 distinct methods. The most common is the well known scalariform method, in which the 2 conjugating filaments have the appearance of a ladder, and the gametes travel in only one direction, so that one filament contains all the zygotes. This has been followed for 20 to 25 pairs of conjugating cells. Zygotes are formed also by lateral conjugation, the contents of one cell passing into the adjoining cell of the same filament. This is accomplished in this species by the bulging of the cell wall away from the septum at one side until there is a small opening left between the 2 cells through which the contents pass. In general appearance the zygotes are like those formed by scalariform conjugation. Usually the cells follow the law laid down by HASSALL (16, p. 34) that 2 males alternate with 2 females. This applies only to those filaments in which lateral conjugation occurs alone. In this species it is frequently accompanied by genuflexions. This method (lateral conjugation) occurs in filaments that are also conjugating in the usual scalariform manner. Zygotes are further formed by true cross-conjugation in which "there is the formation of a perfectly normal zygospore in each of the conjugating filaments." Here,

again, the zygotes have the appearance of those formed by scalariform conjugation. This has been followed for 16 pairs of conjugating cells. In this case they are all in conjugation, and, strangely enough, there are 8 zygotes in each filament. These filaments are shown schematically in fig. 1 of pl. XXV, and in part in fig. 1 of pl. XXIV. This is the only pair of filaments with any considerable number of zygotes thus far found which shows the same number in each of the conjugating filaments. A glance at the plates will emphasize the fact that, in general, there is no such regular order. Whole filaments would probably shed further light upon the occurrence. Moreover, filaments are found frequently in which both true cross-conjugation and lateral conjugation occur.

Much care is needed in the study of cross-conjugation, as there are many chances for error. As previously stated, there are combinations of scalariform and lateral conjugation that at first appear to be cases of true cross-conjugation. The writer has used the utmost care and has been compelled to discard a number of slides that at first were thought to show cross-conjugation. Only such cases as have complied with the following rules have been regarded as in cross-conjugation: (1) zygotes must occur in both filaments, the swelling of the egg cell is insufficient evidence; (2) the connecting tube must be visible; (3) the male cell must be empty; (4) end cells must be discarded unless the preceding conditions are met, since lateral conjugation may have occurred.

Slides have been permanently mounted in glycerine and from these the microphotographs of pl. XXIV were taken. They are made at 225 diameters. The plate shows the original photographs, and no "retouching" has been done either on the plates or the prints. Fig. 1 was made from a plain glycerine mount; while the others were from slides stained either with iodine or Magdala red in order that the cell walls might be made a little clearer. Figs. 2 and 6 show several filaments conjugating with each other. In the other figures only 2 filaments are involved. These are, however, very clear cases.

Schematic drawings have also been made (pl. XXV). In these the writer has laid the filaments parallel on the paper, regardless

of their twisting and turning on the slide. In each case, however, he has retained all the cells, whether conjugating or not, from the first to the last pair of conjugating cells in the filaments. When 3 or more filaments were found conjugating, they have been laid parallel also, and as nearly as possible in their relative position. The writer has adopted the schematic method in this paper in order that the reader may grasp more easily the relative position of the empty cells, unchanged cells, and the zygotes. Diagrammatic drawings can be followed more readily than the windings of the camera drawing, as may be seen by a comparison of fig. 1, pl. XXIV, with fig. 1, pl. XXV. The former is a microphotograph of a portion of the pair of conjugating filaments schematically represented in the latter. Furthermore, the diagrammatic method consumes considerably less space.

Two of these slides were exhibited at the meeting of the North Carolina Academy of Science in May 1915, where they were observed by a number of botanists. Other slides were sent to Professor G. S. WEST, who has referred it to *S. inflata*.

The species of *Spirogyra* under discussion is single banded, with 2 or 3 turns of the band, and has the cell membrane replicate at the ends. The length of the vegetative cell is about 80 μ , and the width about 15 μ . The zygote cell is swollen on both sides. The zygote length is about 43 μ and the width about 28 μ ; zygote oval, considerably pointed, brown at maturity. In these characters it follows closely the descriptions for *Spirogyra inflata*. On the other hand, it is to be noted that the connecting tubes are always put out by the male cell and fuse directly with the cell wall of the female cell. This is true regardless of which filament furnishes the male gamete, and would indicate that the sex character was present even before the conjugation tube was put forth.

This species differs also from *S. inflata* in the phenomenon of cross-conjugation, which has not been ascribed to it by any modern work available to the writer. LOTSY (23, p. 198) states that there is a difference between the male and female filaments of *S. inflata*, which can signify scalariform conjugation only. WOLLE (31) asserts that conjugation may be either lateral or scalariform. DETONI (12, p. 766) gives *Conjuguee renflee* of VAUCHER (citing VAUCHER,

pl. V, fig. 3) as synonymous with *S. inflata*, and, although VAUCHER figures it in cross-conjugation (pl. I, fig. 4), DETONI merely states that conjugation is often lateral. Since *S. inflata* has been under observation for so long, and these conditions have not been recognized as characters, it would seem that we must either form a new species for this plant or include these conditions in the description of *S. inflata*. The writer is opposed to the multiplication of species, but these are such distinct characteristics that plants showing them should, it would seem, be classed separately. Final decision in this matter, however, must be reserved until the writer or someone else has had opportunity for further investigation.

The occurrence of this species presents some new problems in the general theory of sex as it applies to the filament of *Spirogyra*. The work on the cytology of this genus has not been entirely satisfactory, owing to the difficulty of staining and counting the chromosomes. CHMIELEWSKI (7), in 1890, saw evidences of reduction but was unable to count the chromosomes (JOHNSON 20). In 1899 KLEBAHN (21) followed the reduction in the desmids, and 12 years later TRÖNDLE (27) succeeded in counting the chromosomes in *Spirogyra* and found that a reduction takes place in the germination of the zygotes. He further found that 4 nuclei were formed, 3 of which degenerate, while "the fourth remains as the nucleus of the single embryonic plant." Evidently the sex factors are separated, and one or the other of them is thrown out in this reduction, since a filament wholly of one sex results.

In the case of lateral conjugation, however, it would seem that reduction cannot take place in the zygote, as both sexes are present in the filament. Moreover, it would seem that reduction takes place in the divisions just preceding reproduction. This may have occurred in the last division before conjugation. Let

♂	♀
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 represent a cell which upon division separates the male and female factors

♂	♀
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. These conjugating would give alternate zygotes and empty cells. In this case conjugation is assumed to take place between gametes derived from the same mother cell. If, however, they should so divide throughout the filament that the male of one mother cell should adjoin the female from another, we should have the alternation of male and female, so that con-

jugation might take place between daughter cells of different mother cells $\begin{bmatrix} \delta & \varnothing & \delta & \varnothing \end{bmatrix}$. But if they should so divide that the female of one mother cell adjoins the female of another, we should have this occurrence $\begin{bmatrix} \delta & \varnothing & \varnothing & \delta \end{bmatrix}$, which is very characteristic of lateral conjugation. HASSALL (16), observing such a phenomenon, stated that in lateral conjugation there were always 2 empty cells separated by 2 zygotes. WEST (29, pl. V, figs. 72, 73) also figures this condition. WOLLE (31) makes a similar statement which, however, is not borne out by his plates, since in several cases he has described as lateral conjugation conditions which according to his own figures are manifestly aplanospore formations (WOLLE 31, pl. 132, figs. 5, 8; pl. 133, fig. 1; pl. 134, fig. 1).

This characteristic appearance may be brought about, however, by another method of division. If the cells adjoining should so divide that the male and female elements alternate $\begin{bmatrix} \delta & \varnothing & \delta & \varnothing \end{bmatrix}$, and if this were followed by the subsequent division of all the cells, a filament would be produced which upon conjugation would contain 2 zygotes alternating with 2 empty cells $\begin{bmatrix} \delta & \delta & \varnothing & \varnothing & \delta & \delta & \varnothing & \varnothing \end{bmatrix}$. If, however, the division should so occur that male adjoined male and female adjoined female $\begin{bmatrix} \delta & \varnothing & \varnothing & \delta \end{bmatrix}$, then a further division would produce a filament containing 4 consecutive males and 4 consecutive females $\begin{bmatrix} \delta & \delta & \varnothing & \varnothing & \varnothing & \varnothing & \delta & \delta \end{bmatrix}$.

Assuming that reduction has been retarded until just previous to reproduction in lateral conjugation, it would be possible for us to have an alternation of empty cell and zygote or an alternation of 2 empty cells and 2 zygotes. If these filaments should cross-conjugate (and there is no reason why they should not), they might produce results such as diagrammed in pl. XXV, figs. 9, 13; or pl. XXIV, fig. 3. The greatest number of consecutive zygotes would be 2. Assuming further that the second division has taken place, producing 4 nuclei, as usually occurs in the formation of sex cells, the greatest number of consecutive zygotes would be 4.

These numbers cannot explain the conditions figured on pl. XXV, figs. 1, 2, 3, or those of pl. XXIV, figs. 1 and 4, and many others

which the writer has observed but not diagrammed, since cases in which there are more than 4 consecutive zygotes or empty cells show that division has occurred once or more after reduction. Here, then, is a distinct and characteristic difference between lateral and cross-conjugation. In lateral conjugation there can be no further cell division after reduction is complete, but in cross-conjugating filaments there may be. A study of the plates shows that in the case of fig. 2, pl. XXV, division must have occurred 3 times subsequent to reduction in part of the cells at least, in order to produce the 11 consecutive males there shown.

From the foregoing it would seem that the phenomenon of cross-conjugation lies between lateral and scalariform and partakes of some of the characters of each. Like the former, the reduction does not occur in the zygote, but is retarded, and none of the potential gametes is lost. Like the latter, division continues after reduction has taken place. For these reasons it would seem that the filament of *Spirogyra*, in this species and in those with lateral conjugation at least, must be homologized with the sporophyte of higher plants. With these facts as a basis, the following conclusions seem to be justified:

1. Bisexuality of the filament does occur in certain species of *Spirogyra*, but not necessarily in all species.
2. Reduction may occur in the zygote, in which case a filament wholly of one sex arises, or reduction may occur just previous to reproduction, in which case none of the nuclei degenerates, and filaments of a bisexual nature are produced, which would conjugate either laterally or by cross-conjugation.
3. Cell division may take place subsequent to reduction, some cases showing 3 divisions, and this is an essential difference between lateral and cross-conjugation, since the latter may continue cell division after reduction is complete but the former apparently does not.
4. The filament of *Spirogyra*, in this species and those with lateral conjugation, is homologous with the sporophyte of higher plants.

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EXPLANATION OF PLATES XXIII-XXV

PLATE XXIII

FIG. 1.—*Conjuguee majeure* (princeps), in cross-conjugation; copy VAUCHER, pl. IV, fig. 3; *Spirogyra nitida*.

FIG. 2.—*Conjuguee portiquis* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 1; *Spirogyra porticalis* (Meull.) Cleve.

FIG. 3.—*Conjuguee condensae* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 2; *Spirogyra condensata* (Vaucher) Kütz.

FIG. 4.—*Conjuguee renflee* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 3; *Spirogyra inflata* (Vaucher) Rabenh.

FIG. 5.—*Spirogyra gracilis* in cross-conjugation; copy West (29) pl. V, fig. 81.

FIG. 6.—*Zygnema intermedium* HASSALL, in cross-conjugation; copy HASSALL (17) pl. 38, fig. 8; *Spirogyra Weberi* Kütz.

FIG. 7.—*Spirogyra protecta* and *S. majuscula* hybridizing; claimed to be in cross-conjugation; copy of BESSEY (4).

FIG. 8.—*Zygnema orbiculare* HASSALL, figured in cross-conjugation; copy HASSALL (17) pl. 19, figs. 1, 2; *Spirogyra maxima* (Hass.) Wittr.

FIG. 9.—Schematic drawing of "false cross-conjugation" as observed by the writer.

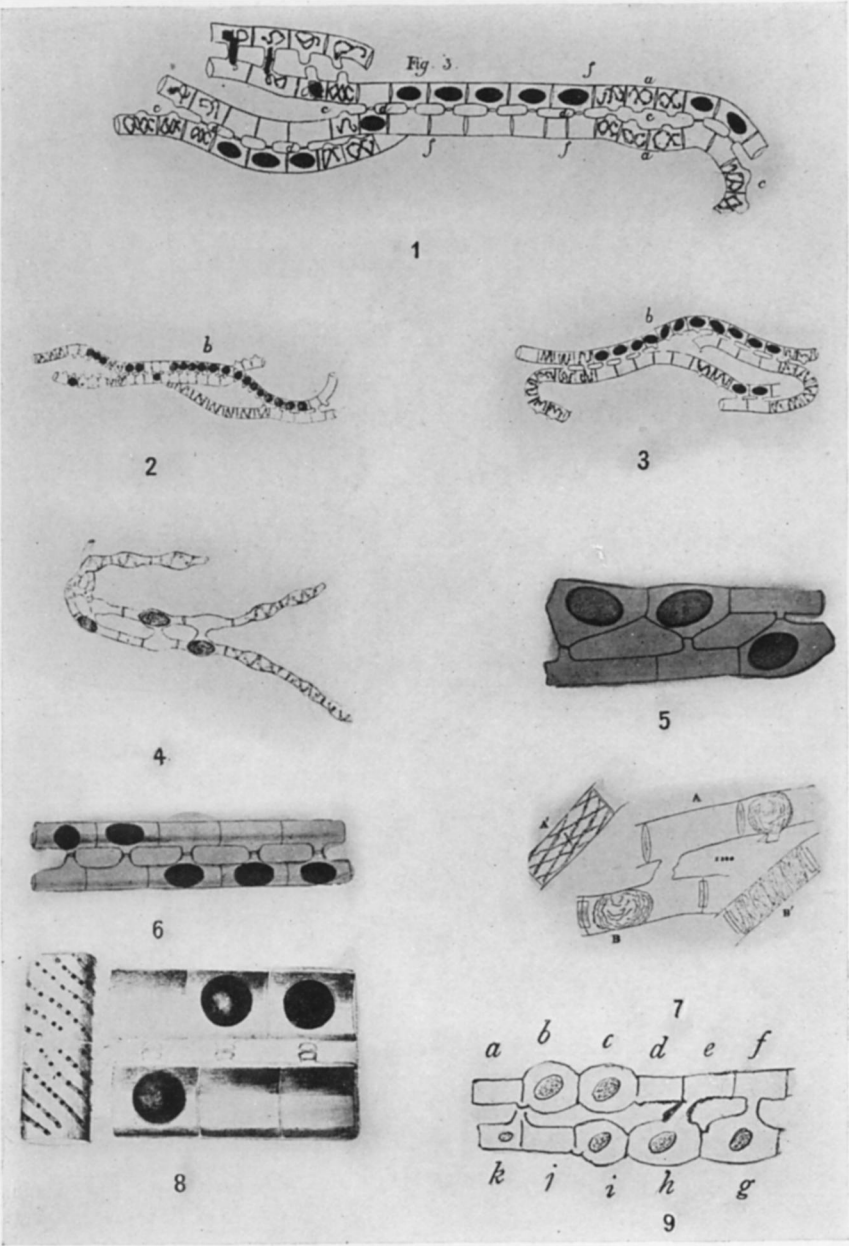
PLATE XXIV

FIG. 1.—Microphotograph of material mounted in glycerine and unstained; $\times 225$.

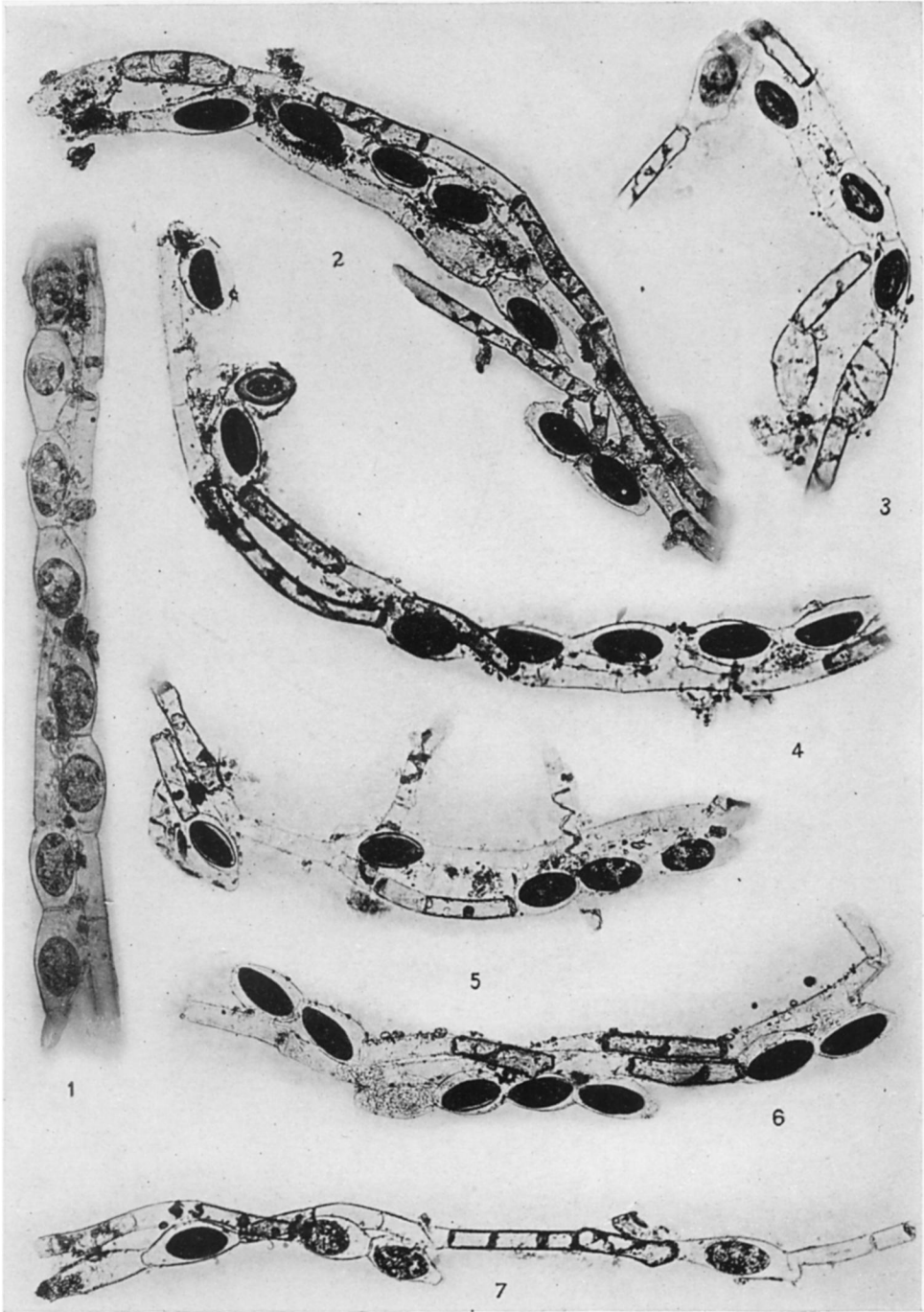
FIGS. 2-7.—Microphotographs of material mounted in glycerine, and stained with iodine and Magdala red; $\times 225$.

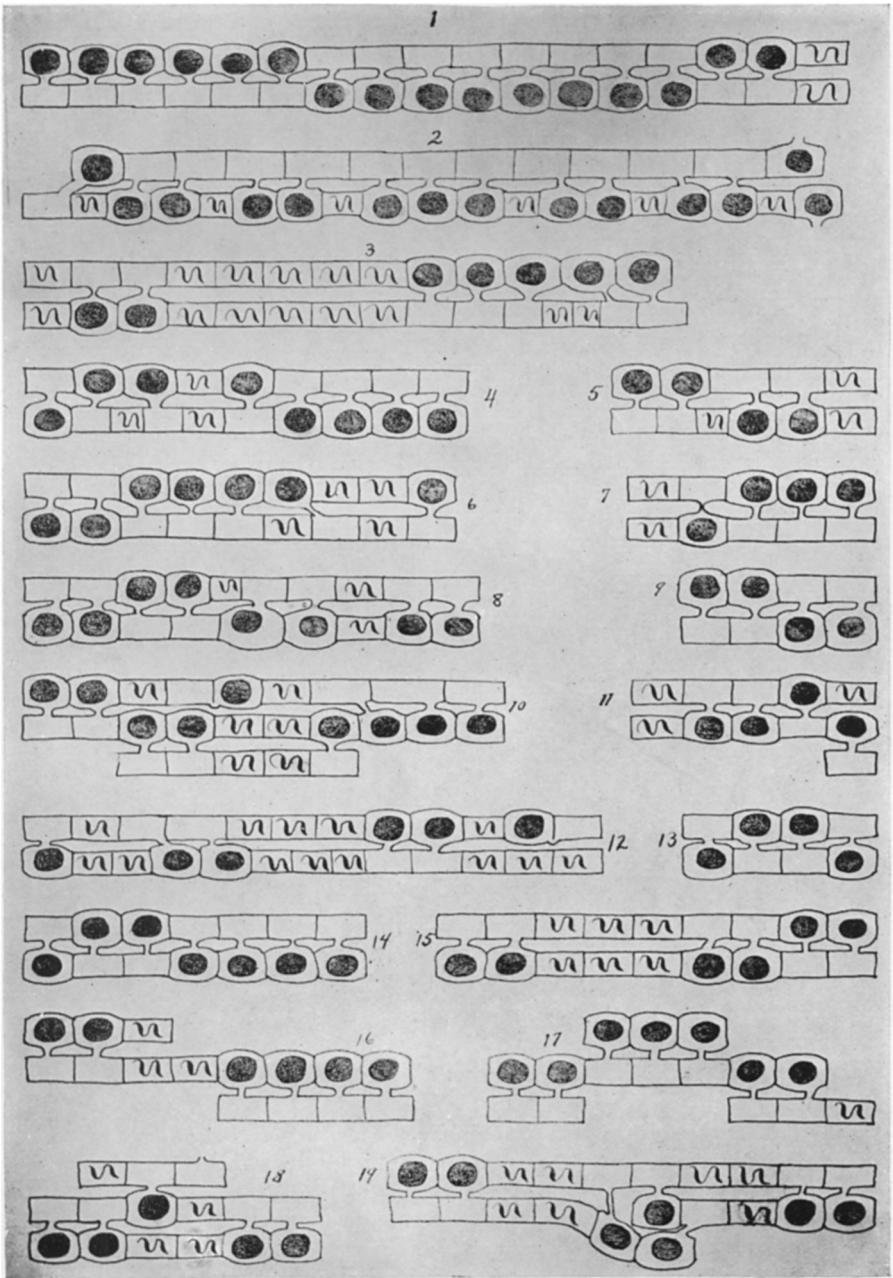
PLATE XXV

FIGS. 1-19.—Schematic drawings of cases of cross-conjugation observed by the writer.



CUNNINGHAM on SPIROGYRA





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