Harrison, J. W. Heslop (1920), "Genetical Studies in the Moths of the Geometrid Genus *Oporabia* (*Oporinia*) with a Special Consideration of Melanism in the Lepidoptera," 'Journ. Gen.,' vol. 9.

Harrison, J. W. Heslop (1922), "The Egg-laying Instincts of *Rhodites rosæ* and Certain Deductions therefrom," 'Vasculum,' vol. 8.

Harrison, J. W. Heslop, and Garrett, F. C. (1926), "The Induction of Melanism in the Lepidoptera and its Subsequent Inheritance," 'Roy. Soc. Proc.,' B, vol. 99.

Harrison, J. W. Heslop (1926), "Miscellaneous Observations on the Induction, Incidence, and Inheritance of Melanism in the Lepidoptera," 'Entomologist,' vol. 59.

Harrison, J. W. Heslop (1926), "Further Experiments on the Egg-laying Instincts of *Rhodites rosæ*," 'Vasculum,' vol. 13.

Houard, C. (1908–13), "Les Zoocécidies des Plantes d'Europe et du Bassin de la Méditerranée." Paris.

---

*Experiments on the Physiology and Genetics of the Smut Fungi. —Hyphal-Fusion.**

By Sydney Dickinson, Rothamsted Experimental Station, Harpenden.

(Communicated by Sir John Russell, F.R.S.—Received November 27, 1926.)

[Plate 10.]

I. *Introduction.*

In recent years attention has been devoted to the study of the Smut Fungi, and their cytology is now fairly well known. The results of previous observations on the two Smuts used in the following experiments may be briefly summarised:—The Chlamydospore in both species is uni-nucleate, and on germination a promycelium is formed into which the nucleus passes. There it divides into two, and the two daughter nuclei pass, one to each end of the promycelium, and there divide, so forming four nuclei between which cross walls are then formed (see text-fig. 1, A–E, p. 130). After this stage the behaviour is governed largely by the environmental conditions, either one or more sporidia are budded off from each segment or clamp connections are formed between pairs of segments. (Text-fig. 1, F–G.) No linear order is apparent in these unions, all possible combinations having been observed; and the nucleus and cytoplasm of one segment passes into the other through the clamp connection.

* This work was carried out in the Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts, with the aid of a grant from the Ministry of Agriculture and Fisheries.

Again, no crossing or changing over in position of the nuclei in the unsegmented promycelium has been seen. It has been presumed therefore that the nuclei in the segments, if numbered from the apex, 1, 2, 3, 4, are in pairs, 1 and 2 being the product of one nucleus, 3 and 4 the product of the other. This has been corroborated in the present work.

Kniep (1) by isolating sporidia at random from a mass of germinating chlamydospores showed that such sporidia could be arranged in two groups, the members of the first, called "A," conjugating with those of the second called "B." No sporidium would conjugate with another of its own group. The numbers in each group were approximately equal, and, from this and the facts already cited, it was deduced that the segregation of "sex" takes place in the second nuclear division in the promycelium.

That two "sexes" are present in many of the Smuts has been shown by Bauch (2); and in one fungus, *Ustilago longissima*, he has described a tri-sexual condition. The sporidia, isolated at random from a mass of germinating chlamydospores of this species, could be arranged in three groups, the members of which, while not conjugating within the group to which they belonged, would conjugate with any from the other two groups.

Kniep (3) has observed conjugation between the germ tubes of sporidia from a number of species of Smuts, and this has also been seen during the present work. By adding conidia from a culture of known "sex" to germinating chlamydospores and observing the conjugations which take place, Kniep (3) has also demonstrated that there is no regularity of position of the "sexes" in the promycelium.

Previous authors have described clamp connections between segments of the promycelium and conjugation between germ tubes of sporidia. In the present work union between the hyphæ of two mycelia is described, analogous to the reactions of the + and − strains of the Phycomycetes (Blakeslee, 4). This behaviour has been used for the determination of "sex." The Smuts used in the experiments were Covered Smut of Oats, *Ustilago levis*, and Covered Smut of Barley, *Ustilago Hordei*.

II. *Technique.*

Chlamydospores were placed on 1 per cent. beef extract agar films on cover slips kept in moist Petri dishes and left in incubators for 12 or more hours at various temperatures. As soon as the spores had germinated and each segment of the promycelium had produced one sporidium, these four sporidia were isolated in turn by means of the "Dickinson Isolator" (5) and

transferred to beef extract agar slopes in test tubes. Each tube was given a fractional number, whose numerator signified the parent chlamydospore, and whose denominator signified the position of the segment on the promycelium from which the particular sporidium had been removed; the segments were numbered from the apex 1, 2, 3, 4. Thus CSOK52/1 means Covered Smut of Oats, Kimpton (specimen and origin); 52 the number of the chlamydospore, and /1 the sporidium isolated from the apical segment of the promycelium.

The cytological observations were made on material which had been germinated or grown on nutrient agar on cover slips or in Petri dishes, and which had been affixed to microscope slides in the following manner:—A thin slice of agar, on the surface of which the mycelium was growing, was cut out of the Petri dish with a scalpel. The mycelium was killed by exposure to osmic acid vapour for about five minutes. The slice was then allowed to dry for 15–30 minutes, exposed to the air of the room, and afterwards inverted on to a microscope slide smeared with fresh egg albumen. The slide was immediately put into strong Flemming's solution for half an hour. After washing in distilled water the specimen was decolorised with hydrogen peroxide and then stained. The stain finally adopted was Heidenhain's hæmatoxylin. After staining the agar was lifted off with a scalpel, this requiring considerable care lest the mycelium be removed at the same time; in delicate cases the agar was removed with the aid of the Isolator. The hyphæ were thus left fastened to the slide in the same relative positions as they had occupied when growing. After de-staining, the transference from water to alcohol was carried out gradually (5 per cent. stages) to prevent shrinkage of the hyphal contents.

Where special stages of development were required, likely hyphæ or conidia were moved from the main culture mass to a fresh part of the film, and there watched; or put into contact with other hyphæ similarly isolated, and, at the particular stage desired, the cultures were treated as described above. This process allowed of the fixing and staining of specific hyphæ whose development had been followed.

III. *Observations on Living Material.*

A. Hyphal-Fusion.—The mycelia produced from sporidia isolated from segments of a promycelium, grow mainly on the surface of the nutrient agar and consist of secondary sporidia (or conidia) and hyphæ, the proportions of which vary according to the medium on which they are growing. The conidia increase by budding, or may give rise to hyphæ. The hyphæ grow at their apices, cutting off behind them cells which may or may not have contents. The cells with contents may produce conidia or new hyphæ.

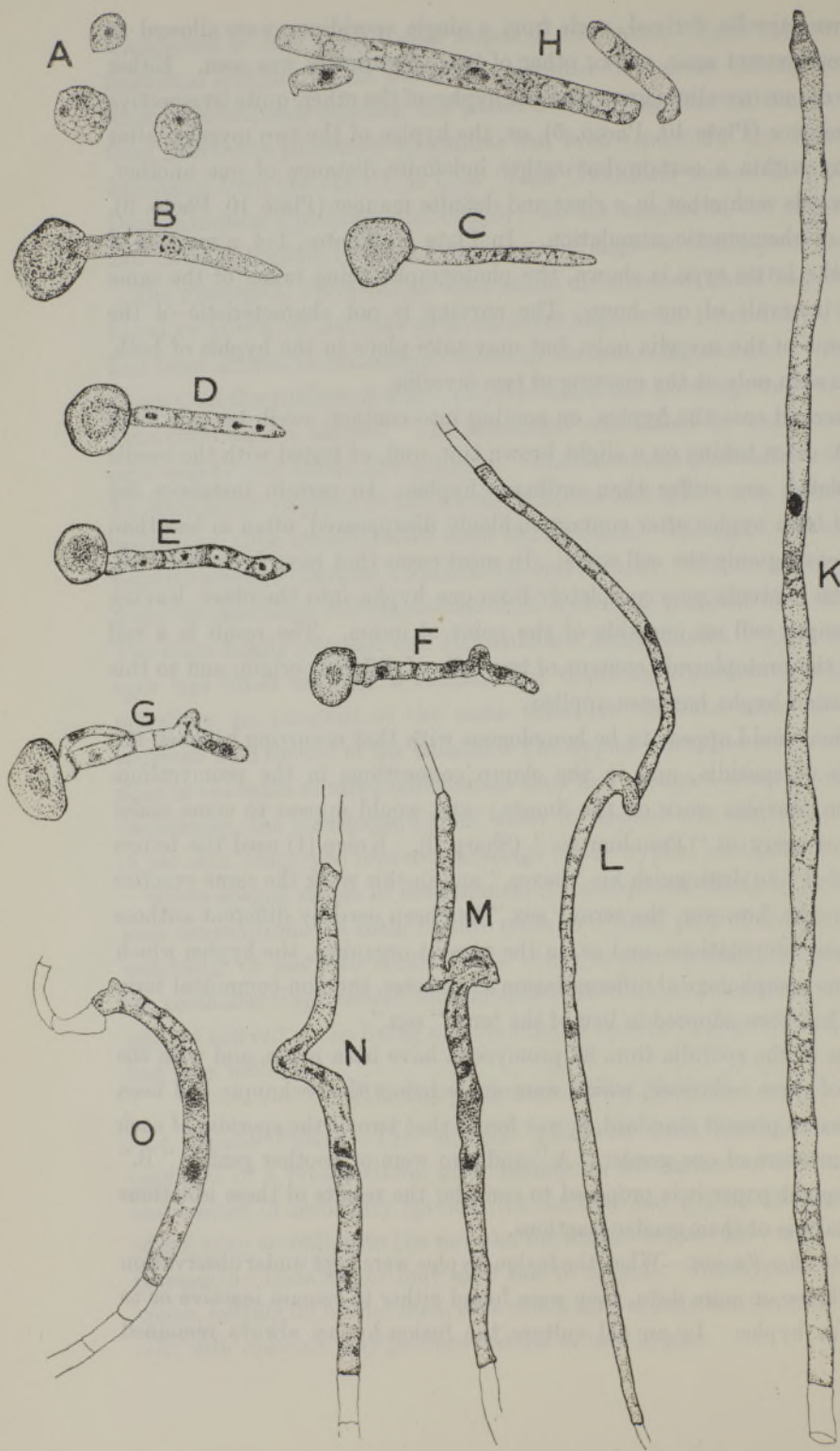
When two mycelia derived, each from a single sporidium, were allowed to meet on beef extract agar, one or other of two appearances was seen. Either the hyphæ of one mycelium grew past the hyphæ of the other, quite irrespective of their presence (Plate 10, Photo. 5), or, the hyphæ of the two mycelia, after approaching within a certain, but rather indefinite distance of one another, curved towards each other in a clear and definite manner (Plate 10, Photo. 3), suggestive of chemotactic stimulation. In Plate 10, Photos. 1–4, a meeting of hyphæ of the latter type is shown, the photographs being taken of the same hyphæ at intervals of one hour. The curving is not characteristic of the hyphæ of one of the mycelia only, but may take place in the hyphæ of both. It has been seen only at the meeting of two mycelia.

In this second case the hyphæ, on coming into contact, swell, become more translucent, often taking on a slight brown tint, and, as tested with the needle of the Isolator, are stiffer than ordinary hyphæ. In certain instances the contents of both hyphæ after contact suddenly disappeared, often in less than a second, leaving only the cell walls. In most cases that have been observed, however, the contents pass completely from one hypha into the other, leaving only the empty cell on one side of the point of union. The result is a cell containing the protoplasmic content of two cells of different origin, and to this the term fusion-hypha has been applied.

This fusion would appear to be homologous with that occurring between the germ tubes of sporidia, and in the clamp connections in the promycelium described in previous work on the Smuts; and would appear to come under Winkler's category of "Pseudomixis" (Sharp 6). Kniep (1) used the letters "A" and "B" to distinguish his "sexes," and in this work the same practice is followed. As, however, the term "sex" has been used by different authors with different connotations, and as, in the present organism, the hyphæ which fuse show no morphological differentiation as gametes, the non-committal term "gender" has been adopted in lieu of the term "sex."

Isolations of the sporidia from 82 promycelia have been made, and with the exception of three isolations, which were made before the technique had been improved to its present standard, it was found that two of the sporidia of each promycelium were of one gender "A" and two were of another gender "B." In a subsequent paper it is proposed to consider the results of these isolations with an analysis of their gender reactions.

B. Growth after Fusion.—When the fusion-hyphæ were kept under observation for one to three or more days, they were found either to remain inactive or to grow out as hyphæ. In an old culture the fusion-hyphæ always remained



Text-fig. 1.—All drawings in this figure have been made with the aid of a camera lucida, using a Leitz 1/10*u* oil-immersion objective and periplan : oculars 6, 10, 12. Cells drawn in outline are those with no contents.

A.—Chlamydospores of Covered Smut of Oats with the spore coat removed, showing uni-nucleate condition. × 890.

B–E.—Stages in the germination of the chlamydospore with the formation of a promycelium, and the divisions resulting in four nuclei. × 890.

F–G.—Clamp connections being formed between the uni-nucleate segments of the promycelium. × 890.

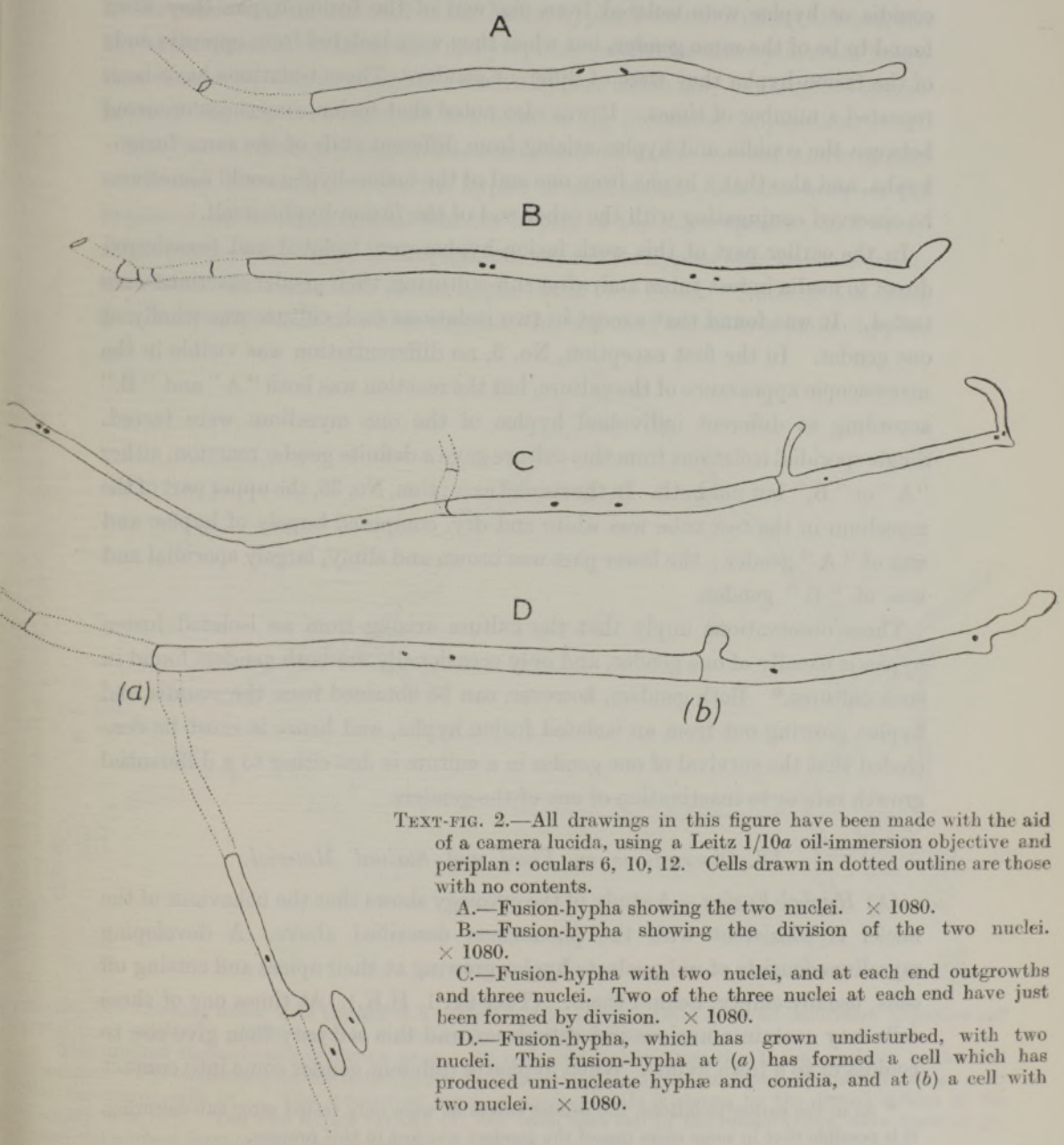
H–K.—Uni-nucleate hyphæ and conidia, characteristic of the mycelia arising from single sporidial isolations. × 1340.

L.—Two hyphæ of different gender in contact with each other, showing the characteristic curving. × 890.

M–N.—Two hyphæ, after fusion, showing both the nuclei in one of the hyphæ. × 890.

O.—Fusion hypha showing the contents of both the original hyphæ on one side of the point of fusion. × 890.

inactive, but if they were moved by the Isolator to a fresh part of the agar, or if, on the other hand, they had been formed in a young culture, they proceeded to grow in one of two ways. Sometimes they continued to grow, branching occasionally and resembling in every way the original fusion-hypha, and leaving



Text-fig. 2.—All drawings in this figure have been made with the aid of a camera lucida, using a Leitz 1/10*a* oil-immersion objective and periplan: oculars 6, 10, 12. Cells drawn in dotted outline are those with no contents.

A.—Fusion-hypha showing the two nuclei. × 1080.

B.—Fusion-hypha showing the division of the two nuclei. × 1080.

C.—Fusion-hypha with two nuclei, and at each end outgrowths and three nuclei. Two of the three nuclei at each end have just been formed by division. × 1080.

D.—Fusion-hypha, which has grown undisturbed, with two nuclei. This fusion-hypha at (*a*) has formed a cell which has produced uni-nucleate hyphæ and conidia, and at (*b*) a cell with two nuclei. × 1080.

behind them empty cells and, from time to time, cells with contents. After several days, in all such specimens observed, they either died or stopped growing. In other cases conidia and hyphæ were formed at one or at both ends of the isolated fusion-hypha, and these eventually produced a new mycelium. When conidia or hyphæ were isolated from one end of the fusion-hypha they were found to be of the same gender, but when they were isolated from opposite ends of the fusion-hypha they were of different gender. These isolations have been repeated a number of times. It was also noted that fusion sometimes occurred between the conidia and hyphæ arising from different ends of the same fusion-hypha, and also that a hypha from one end of the fusion-hypha could sometimes be observed conjugating with the other end of the fusion-hypha itself.

In the earlier part of this work fusion-hyphæ were isolated and transferred direct to media in test tubes and, after sub-culturing, their gender reactions were tested. It was found that except in two isolations each culture was wholly of one gender. In the first exception, No. 5, no differentiation was visible in the macroscopic appearance of the culture, but the reaction was both "A" and "B," according as different individual hyphæ of the one mycelium were tested. Single sporidial isolations from this culture gave a definite gender reaction, either "A" or "B," but not both. In the second exception, No. 39, the upper part of the mycelium in the test tube was white and dry, composed largely of hyphæ and was of "A" gender; the lower part was brown and slimy, largely sporidial and was of "B" gender.

These observations imply that the culture arising from an isolated fusion hypha is usually of one gender, and only occasionally are both genders found in such cultures.* Both genders, however, can be obtained from the conidia and hyphæ growing out from an isolated fusion hypha, and hence it must be concluded that the survival of one gender in a culture is due either to a differential growth rate or to inactivation of one of the genders.

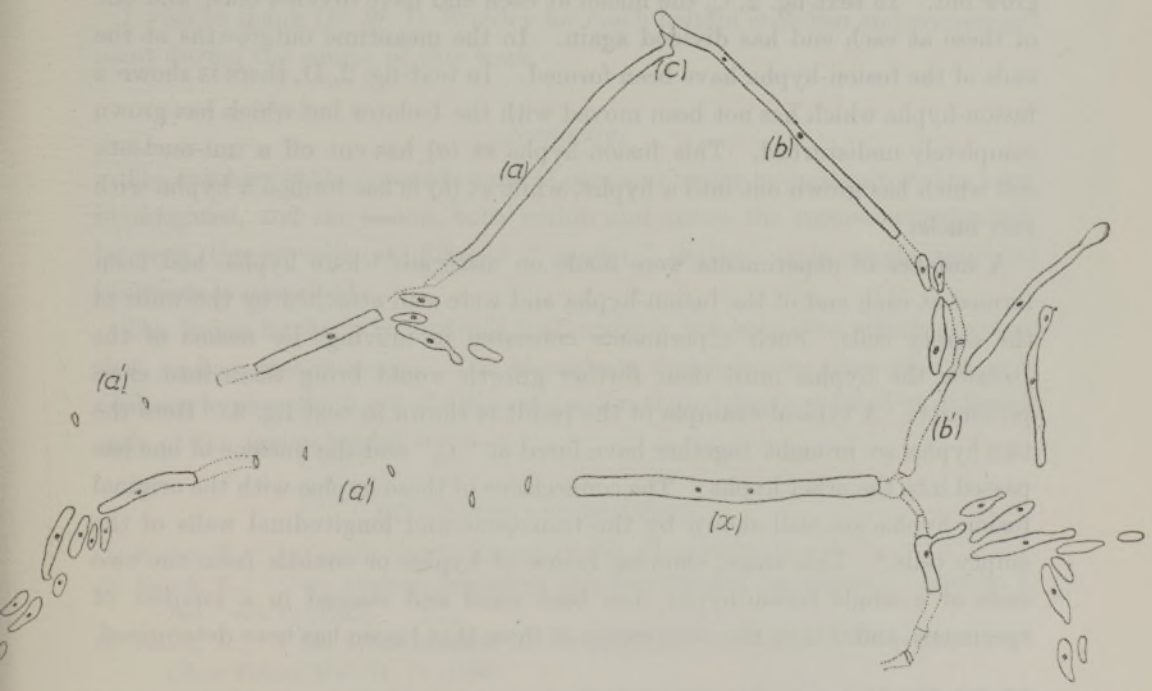
IV. *Observations on Fixed and Stained Material.*

(A) *Hyphal-Fusion.*—A study of the cytology shows that the behaviour of the nuclei is consistent with the phenomena described above. A developing mycelium consists of uni-nucleate hyphæ growing at their apices and cutting off cells, usually empty, behind them. (Text-fig. 1, H.K.) At times one of these cells may contain a nucleus and cytoplasm, and this cell may then give rise to conidia or to a fresh hypha. When hyphæ of different gender come into contact

* As in the earlier isolations, the gender reactions were only tested after sub-culturing, it is possible that in some cases one of the genders was lost in this process.

they either fuse immediately or twine or coil once or twice and then fuse at their tips (text-fig. 1, L.). As soon as the portion of hyphal wall separating the cytoplasms has disappeared, the contents pass from one hypha into the other and the two nuclei approach one another and lie side by side (text fig. 1, M.N.O.). In the meantime the cell wall of the fusion hypha becomes slightly thicker, the hypha increases in diameter and its cytoplasm retains hæmatoxylin stain longer than that of the ordinary hypha. Such a hypha, which may be called a " fusion-hypha," has only been observed as a result of the process of conjugation.

This type of fusion of hyphæ has been observed in the covered smuts of oats and barley, not only between the hyphæ of each species, but also between the hyphæ of one of these species and the hyphæ of the other species ; and every observation of this type of conjugation, where two hyphæ only are concerned, has shown the immediate fusion-hypha with two nuclei. Nothing has been seen in the preparations which suggests the fusion of nuclei in a fusion-hypha.



TEXT-FIG. 3.—Drawing made with the aid of a camera lucida, using a Leitz 1/10*a* oil-immersion objective and periplan : ocular 10. Cells drawn in dotted outline are those with no contents.

The original fusion-hypha is shown at *x*. At each end it has given rise to uni-nucleate hyphæ and conidia. The connection between the hypha (*a*) and the fusion-hypha is shown by the transverse walls of the empty cells (*a'*). The connection between the hypha (*b*) is shown by the dotted outline of the empty cells (*b'*). The two hyphæ (*a*) and (*b*), one from each end of the fusion-hypha, have fused at (*c*), and the nucleus from (*a*) has moved into the hypha (*b*). × 1080.

In certain specimens three or more hyphæ have apparently fused; and in one example of a triple fusion, where the fusion-hypha could be stained, three nuclei were present. This would appear to be the same phenomenon which Kniep (3) has described in living material.

(B) *Growth after Fusion.*—The cells of all the mycelia arising from isolated fusion-hyphæ were uni-nucleate, except that of No. 5, where, in addition, cells, similar to fusion-hyphæ with two and more nuclei, were also present.

Isolated fusion-hyphæ were grown, their development watched, and at the appropriate time they were fixed and stained. The fusion-hypha has been found to be at first bi-nucleate (text-fig. 2, A). Each of these two nuclei of the fusion-hypha divide (text-fig. 2, B), and two of the products of this double division remain in the centre, while one passes to each end of the hypha; a wall is then formed on either side of the two central nuclei. The single nuclei in the new cells at either end divide frequently and uni-nucleate conidia and hypha grow out. In text-fig. 2, C, the nuclei at each end have divided once, and one of these at each end has divided again. In the meantime outgrowths at the ends of the fusion-hypha have been formed. In text-fig. 2, D, there is shown a fusion-hypha which has not been moved with the Isolator but which has grown completely undisturbed. This fusion-hypha at (*a*) has cut off a uni-nucleate cell which has grown out into a hypha, while at (*b*) it has formed a hypha with two nuclei.

A number of experiments were made on materials where hyphæ had been formed at each end of the fusion-hypha and were still attached by the walls of the empty cells. Such experiments consisted in moving, by means of the Isolator, the hyphæ until their further growth would bring them into close proximity. A typical example of the result is shown in text-fig. 3. Here the two hyphæ so brought together have fused at "C," and the nucleus of one has passed into the other hypha. The connections of these hyphæ with the original fusion-hypha are still shown by the transverse and longitudinal walls of the empty cells.* This stage, showing fusion of hyphæ or conidia from the two ends of a single fusion-hypha, has been fixed and stained in a number of specimens, and it is on the observation of these that fusion has been determined.

* In the present condition of this slide, the cross walls only can be seen in the case of one of the hyphæ (*a'*). The reason for this is that the amount of de-staining which is necessary to differentiate the nuclei removes the stain almost entirely from the walls of the empty cells.

V. *Conclusions.*

The conclusions drawn from these observations are as follow :—The fusion of two hyphæ of different gender results in a cell containing two associated nuclei, one of " A " gender and the other of " B " gender. This bi-nucleate cell produces a hypha, under certain conditions, into which the two nuclei pass. After some growth of this hypha the two nuclei divide, and, of the four products of division, one nucleus of " A " gender passes to one end of the hypha, two nuclei, one of " A " gender and one of " B " gender, remain associated in the centre, and the fourth nucleus, of " B " gender, passes to the other end of the hypha. In this way an order A, AB, B, or *vice versa*, is obtained in the hypha. The cells containing single nuclei produce uni-nucleate conidia and hyphæ. Since the single nuclei are of different gender, a new fusion-hypha results when two of the hyphæ from opposite ends of the fusion-hypha are brought together.

I wish to thank Dr. W. B. Brierley for much helpful criticism and encouragement during the course of this work.

Summary.

The cytology of the covered smuts of oats and barley in pure culture has been investigated, and the fusion, both within and across the species investigated, between the mycelia of different " gender " derived from single sporidial isolations is described.

The fusion-hypha is bi-nucleate, and nothing has been seen which suggests that nuclear fusion occurs. The bi-nucleate fusion-hypha gives rise to uni-nucleate hyphæ which are of different gender, these being produced at different ends of the fusion-hypha.

VII.—LITERATURE CITED.

(1) Kniep, H., " Antherenbrand," ' Zeitschr. f. Bot.,' vol. 11 (1919).
(2) Bauch, R., " Über Ustilago longissima und ihre Varietät macrospora," ' Zeitschr. f. Bot.,' vol. 15 (1923).
(3) Kniep, H., " Über Artkreuzungen bei Brandpilzen," ' Zeitschr. f. Pilzkunde,' vol. 5 (Neue Folge), Heft 14, 15 (1926).
(4) Blakeslee, A. F., " Sexuality in Mucors," ' Science, N.S.,' vol. 51, pp. 375–382, 403–409 (1920).
(5) Dickinson, S., " A Method of Isolating and Handling Individual Spores and Bacteria " ' Proc. Roy. Soc. Med.,' vol. xix, pp. 1–4, 1926 (*see also* ' Ann. Bot.,' vol. 40 (1926).)
(6) Sharp, W., ' Introduction to Cytology.' New York, 1926.

DESCRIPTION OF PLATE 10.

All photographs have been taken with a Leitz "Micca" camera, with a No. 6A Leitz objective and a periplan : ocular 10, and enlarged.

PHOTOGRAPHS 1–4.—These are a series of photographs taken at intervals of one hour, showing the meeting of the hyphæ of two mycelia of different gender. The curving (see hyphæ within circle) which is most marked in Photograph 3, is characteristic of such a meeting.

PHOTOGRAPH 5.—This is a photograph of the meeting of hyphæ of two mycelia of the same gender.

The History of the Cytoplasmic Inclusions of the Egg of Ciona Intestinalis (L.) *during Oogenesis and Fertilisation.*

By L. A. HARVEY, M.Sc., A.R.C.S., Assistant in the Department of Natural History and Zoology, University of Edinburgh.

(Communicated by Prof. E. W. MacBride, F.R.S.—Received October 16, 1926.)

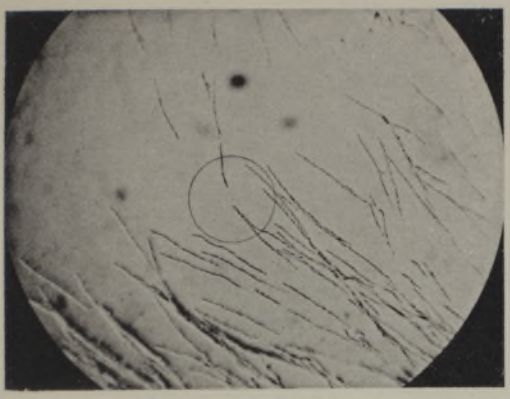
[PLATES 11–13.]

CONTENTS.

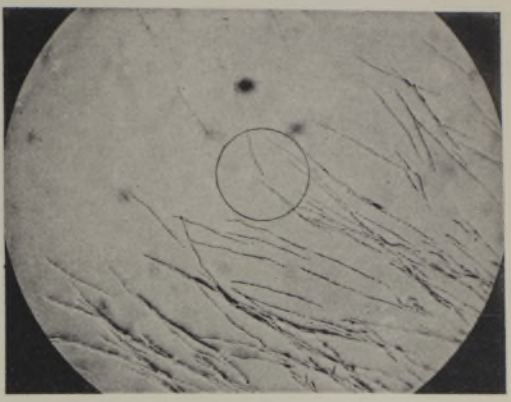
		PAGE
1.	Introduction	136
2.	Literature	137
3.	Material and Methods	140
4.	Mitochondria	141
5.	Golgi Apparatus	145
6.	Yolk Nucleus	148
7.	Test-cells	150
8–10.	Yolk—Cytoplasm–Nucleus	152
11.	Changes after egg has left ovary	155
12.	Discussion	156
13.	Summary	160
14.	Bibliography	161
15.	Description of Plates	162

§1. *Introduction.*

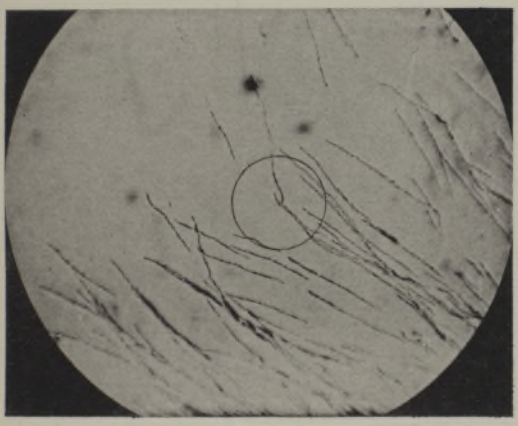
In a previous paper the process of yolk formation in the oogenesis of *Lumbricus terrestris* has been investigated (10). In the present paper the history of yolk formation and of the cytological constituents of the oocyte of *Ciona intestinalis* is traced, and an attempt is made to correlate the functions of these bodies with the metabolism of the cell.



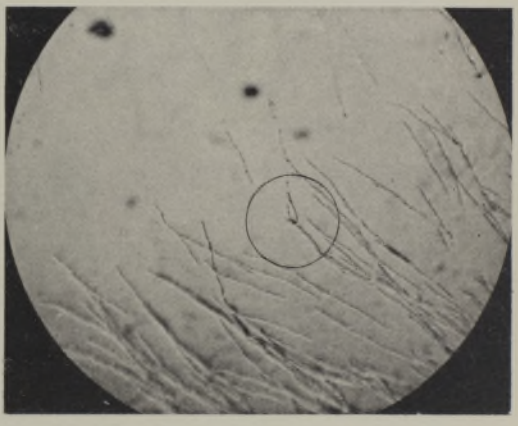
1



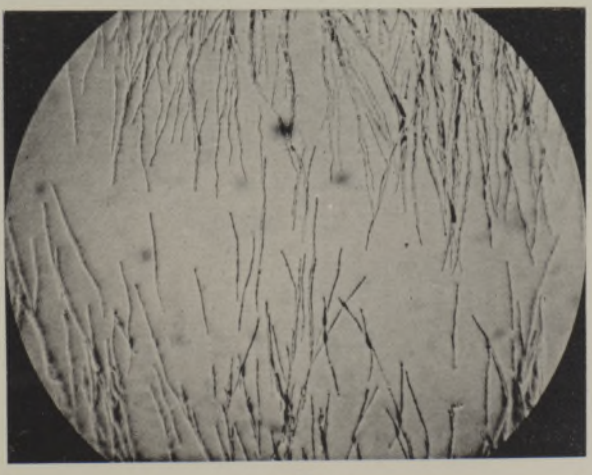
2



3



4



5

London: Stereoscopic Co. Imp.