Nuclear Migrations in Phragmidium violaceum.

BY

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With Plate XVI.

DURING the last ten years much work has been done on the life-history of the Uredineae, and it has brought to light the fact that the development of the aecidiospores is initiated by the formation of binucleated cells at the base of the aecidium.

Blackman (1),[1] in 1904, was the first to show how this took place. He found that in *Phragmidium violaceum* the binucleate condition originated in the 'fertile cell' of the aecidium, and was the result of the migration into it of the nucleus of a neighbouring cell. He considered this to be a case of reduced fertilization, in which a vegetative cell takes the place of a normal male cell. He regarded the spermatia as male cells which had become functionless, and suggested that the sterile terminal cell might represent an abortive trichogyne.

In 1905 Christman (3) investigated the aecidial development of *Phragmidium speciosum*. He found that in this form the binucleate condition is brought about by the gradual breaking down of the walls separating two adjacent fertile cells, so that they fuse and eventually give rise to a row of binucleate aecidiospores. He considered this to be a conjugation of two equal gametes. The spermatia he regarded as gametophytic conidia. This type of fertilization was later interpreted by Blackman and Fraser (2) as a case of reduced fertilization in which two female cells associate. This second type of fertilization is thus brought into line with the earlier one found in *Phragmidium violaceum*.

Since this time a large number of forms have been investigated, and it has been shown in the majority of cases that fertilization takes place by the union of two similar cells. Such a mode of origin of the binucleate condition has been described for *Phragmidium speciosum* (3), *Melampsora Rostrupi* (5), *Phragmidium potentillae canadensis* (5), *Caeoma nitens* (12 and 13), *Triphragmium Ulmariae* (13), *Puccinia transformans* (13), *Puccinia*

[1] The numbers refer to the list of papers given at the end.

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Falcariae (6), *Endophyllum sempervivum* (11), *Melampsora Linii* (8), *Puccinia Claytoniata* (9), *Uromyces Caladii* (3 and 9), *Puccinia violae* (9), *Puccinia angustata* (9), and *Puccinia fusca* (14). So far fertilization in the Uredineae by means of a migrating nucleus has been clearly observed only in *Phragmidium violaceum* (1), and with somewhat less clearness in *Uromyces Poae* (2) and *Puccinia Poarum* (2).

The far more common occurrence of the method of fertilization by the union of similar cells has led to some doubt being thrown on the importance of nuclear migration in the sexual process of this group. Olive (13) found nuclear migrations in *Triphragmium Ulmariae* and *Caeoma nitens*, but held them to be early stages of cell fusions, whilst Christman and Kurssanow, on the other hand, considered them to be pathological phenomena. Professor Blackman accordingly suggested to me the re-examination of the developing aecidium of *Phragmidium violaceum*, especially with a view to ascertain if cell fusions, as well as nuclear migrations, were to be found there.

The material was collected on Leith Hill and in the neighbourhood of Guildford in the spring of 1913 and 1914. Owing to the difficulty of distinguishing between *Phragmidium Rubi* and *Phragmidium violaceum* in the aecidial stage, teleutospores were gathered in the autumn from those blackberry bushes which had previously furnished the material of aecidia. The characters of the teleutospore were those of *Phragmidium violaceum*, Wint., the form investigated by Blackman (1).

Special attention was paid to fixation in view of Christman's suggestion that the nuclear migrations might have been the result of wounding during that process. The young aecidial patches are red in colour and were always cut with a sharp pair of scissors from leaves still on the plant, leaving a margin of green tissue round each infected area. The material was then put direct into the fixative. Flemming's strong solution, diluted with an equal quantity of water, was commonly used, penetration of the fluid being facilitated by the use of a small air-pump, so that the material sank in a few moments. Well-fixed material was also obtained by using in the same way Bouin's picro-formol. As a control, some satisfactory preparations were made by momentarily immersing the material in 30 per cent. alcohol before placing in the fixing fluid; this ensured the quick penetration of the fluid without the use of a pump. Material was also fixed in acetic alcohol.[1]

A very careful and prolonged search has been made for any indications of fusions between fertile cells of the young aecidium, but without success; no case of this type of fertilization was found. Migrations of nuclei, such as were previously described by Blackman, occur regularly. The nucleus invariably passes from a vegetative to a fertile cell; sometimes the cells concerned belong to the same hypha and the nucleus passes from below upwards (Pl. XVI, Fig. 9), and at other times the nucleus comes from a neighbouring

[1] Nuclear migrations were found in material fixed in all these various ways.

hypha and passes in laterally (Fig. 2). In no case was a migration found from one fertile cell to another. In very young aecidia the migrations were found to occur in the middle region of an aecidium, whilst in rather older examples cells of this region were already binucleate, and then the migrations were found only in the cells immediately outside these. Sometimes the nucleus passes through a very small pore in the wall (Figs. 1 and 4), and thus becomes greatly constricted (cf. Blackman (1), Figs. 67, 68). More commonly, however, the pore is rather larger, and the nucleus is only slightly constricted during its passage and corresponds to Blackman's Fig. 66 ; such cases are shown in Pl. XVI, Figs. 2, 3, 5, 6, and 7. In a few cases the hole was of considerable size, as shown in Figs. 8 and 9. When it was found that the nuclei sometimes pass through a large hole on the cell-wall, it seemed unlikely that such holes would be later obliterated, and a careful search was made amongst the older binucleate cells, with the result that a few cases were observed, and two of these are shown in Pl. XVI, Figs. 10 and 11.

The cells from which the nuclei have migrated gradually lose their cytoplasm and become almost or quite empty; they form a fairly conspicuous layer surrounding the bases of the old fertile cells. Pl. XVI, Fig. 13, is a semi-diagrammatic drawing of the middle of a young aecidium, the fertile cells are binucleate, and below them is the layer of empty cells. Part of the same aecidium, but nearer to the periphery, is shown in Fig. 12 where the fertile cells are as yet uninucleate and have no empty cells at their bases. In thick sections of older material the empty cells form a conspicuous layer; Fig. 14 is a semi-diagrammatic representation of such a preparation, showing the young aecidiospores, the binucleate fertile cells, a layer of empty cells, and below that a mass of uninucleate hyphae ramifying in the tissue of the host.

In spite of a careful search, fusion between two fertile cells was never observed, but no less than twenty-eight cases of migration of a vegetative nucleus into a fertile cell were found. It is also important to note, as stated earlier, that in fairly young aecidia the migrations are found to occur only in connexion with the cells immediately peripheral to the central mass of binucleate fertile cells. This special localization of the 'migrations' and the absence of lateral fusion of fertile cells are in themselves sufficient to show that the passage of a vegetative nucleus into a fertile cell is the normal method of origin of the binucleate condition in this form. Migrating nuclei always pass from a vegetative to a fertile cell, and no cases were found of nuclear migrations between fertile cells or between vegetative cells. It may be mentioned that the paraphyses towards the periphery of the aecidium are often multinucleate, and nuclear divisions occur frequently in them.

Christman has put forward a very interesting view as to the morphology of the aecidium and the phylogeny of the group generally. He points out that, from analogy with other groups, the gametophyte generation should be the primitive generation, and therefore that those forms with the simplest sporophyte generation—that is to say, the lepto- and micro-forms—are the most primitive. He regards the various types of spores as homologous, and considers that in the lepto- and micro-group the gametophyte bears the gametes and produces the fusion cell. The point at which the cell fusions occur has receded further and further from the teleutospore, the sporophyte generation becoming more elaborate, till, in the higher groups, an aecidium has been introduced and the eu-forms appear. The 'fusion cell' he regards as the product of two *isogametes* which have formed a zygospore like that of the moulds, but, unlike them, has no resting stage and produces many spores. The spermatia he regards as gametophytic conidia.

The main objections to this view are (1) that it ignores the phenomena described for *Phragmidium violaceum*, assuming that they are due to some pathological cause, and (2) that it offers no adequate explanation of the spermatia.

The observations recorded in this paper show the untenability of the view that nuclear migrations in the aecidium of *Phragmidium violaceum* are pathological in nature. Also one would hardly expect to find conidia which are apparently functionless produced at the same time as the very effective aecidiospores; and, as has been pointed out by Blackman, the relative proportion of nucleus and cytoplasm exhibited by the spermatia is quite out of keeping with what is known of conidia.

The alternative hypothesis is that put forward by Blackman in 1904. He regards the Uredineae as a group in which a great variety of reduced forms occur. He considers that they are derived from an ancestor with a typical sexual process, the male cells being now represented by the spermatia and the female by the 'fertile cells'; these latter were provided with a trichogyne which now possibly exists as a 'sterile' or 'buffer' cell. According to this interpretation, all these Uredineae which have at present been investigated have a reduced type of fertilization, *Phragmidium violaceum* being fertilized by a vegetative instead of a male cell, whilst in *Phragmidium speciosum* reduced fertilization is effected by means of female cells. The two types of fusion found in the Uredineae are thus considered to be heterogamous instead of isogamous, and the group is regarded as showing relationship with the Florideae rather than with the Zygomycetes.

It is, of course, very difficult to decide between these two views. There are at present no data as to whether the heterogamous or isogamous union in the aecidium is the more primitive. If the nuclear migrations of *Phragmidium violaceum* are to be looked upon as reduced in comparison with the isogamous unions which have been described for so many aecidia, then it is still possible to homologize, with Christman, the aecidium and the primary uredospore cell. On the other hand, the fact that the Caeomas of closely allied forms show two very different types of fertilization certainly lends support to the view that both are reduced, being derived probably from an earlier normal sexual process of fertilization by spermatia. If we agree that the peculiar fertilization processes of the aecidium and the existence of the spermatia are sufficient evidence that the more primitive sexual organs are to be found in the aecidium, then we must assume that this type of spore form is the oldest. This is the essential point of Blackman's view as opposed to Christman's. It is not necessary to assume that the eu-forms as they occur at the present day are more primitive than, for example, the brachy- or micro-forms. It is possible that these forms were reduced from a primitive aecidium-bearing form independently of the eu-forms, by the loss of the aecidium and the shifting forwards in the life-history of the point of nuclear association. On the other hand, the complex eu-forms may have developed independently with further elaboration of the life-history, but without loss of the aecidium.

Summary.

1. A re-examination of *Phragmidium violaceum* completely confirms Blackman's observation that fertilization is brought about by the migration of a vegetative nucleus to a fertile cell.

2. No other mode of origin of the binucleate cells was observed.

3. The size of the pore through which the nucleus passes is very variable, sometimes being as much as 3 μ in width. Cells were found in which the pore was visible after the nucleus had migrated through it.

4. A layer of more or less empty cells occurs immediately below the binucleate fertile cells, and is made up of those cells from which the nuclei have migrated.

5. That the nuclear migrations are not pathological in nature is shown by the facts that:

(i) They occur in regular sequence from the middle to the periphery of the aecidium.

(ii) They are not found in the paraphyses at the periphery of the aecidia where the cells are nearer to the wounded surface.

(iii) They are found in material fixed in various ways.

It is with great pleasure that I record my thanks to Professor Blackman for his valuable help and criticism.

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DESCRIPTION OF PLATE XVI.

Illustrating Miss Welsford's Paper on *Phragmidium violaceum*.

Fig. 1. Migration of nucleus. The sterile cell has been cut away. × 1,300.

Fig. 2. Migration of nucleus between the cells of separate hyphae. × 1,300.

Fig. 3. Ditto. × 1,300.

Fig. 4. Migration of nucleus from vegetative cell to fertile cell immediately above it on the same hypha. × 1,300.

Fig. 5. Ditto. × 1,300.

Fig. 6. Migration of nucleus from vegetative cell of one hypha to fertile cell of another. × 1,300.

Fig. 7. Ditto. × 1,300.

Fig. 8. Ditto. In this case the nucleus is passing through a very large pore between two hyphae. × 1,300.

Fig. 9. Ditto. The nucleus passing through a large pore between two cells of the same hypha. × 1,300.

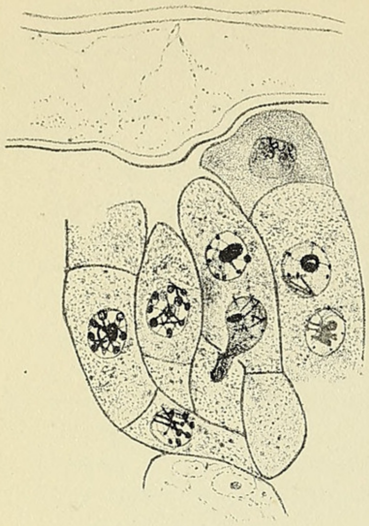
Fig. 10. A binucleate cell showing the pore through which the nucleus has passed. × 1,300.

Fig. 11. A binucleate cell showing the pore through which the nucleus has passed. × 1,300.

Fig. 12. Semi-diagrammatic drawing of a young aecidium (peripheral region). The fertile cells are uninucleate, and no empty cells can be seen. x = host cells. × 500.

Fig. 13. Semi-diagrammatic drawing of the same aecidium as that shown in Fig. 12, but in the median region. Here the fertile cells have become binucleate and empty cells can be seen near their bases. x = host cells; e = empty hyphal cells. × 500.

Fig. 14. Semi-diagrammatic drawing of a nearly mature aecidium, showing the young aecidiospores, the binucleate fertile cells, the layer of empty cells, and the uninucleate hyphae ramifying amongst the host cells. × 500.



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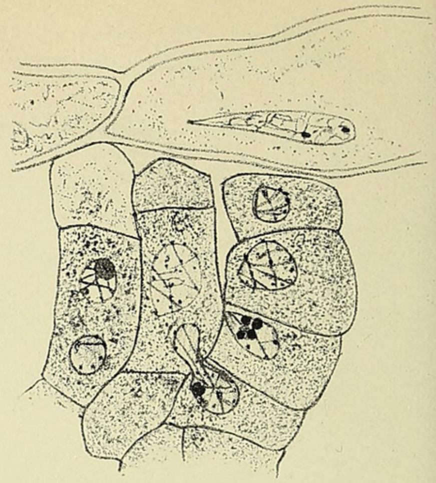

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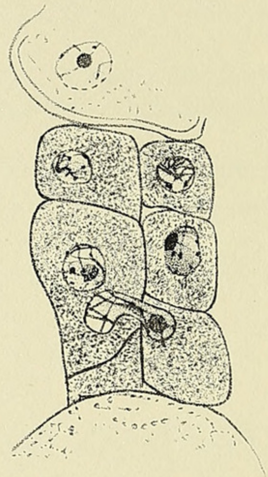

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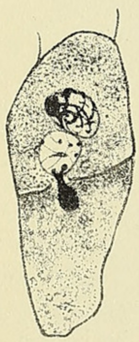

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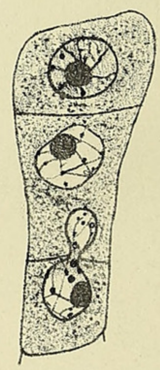

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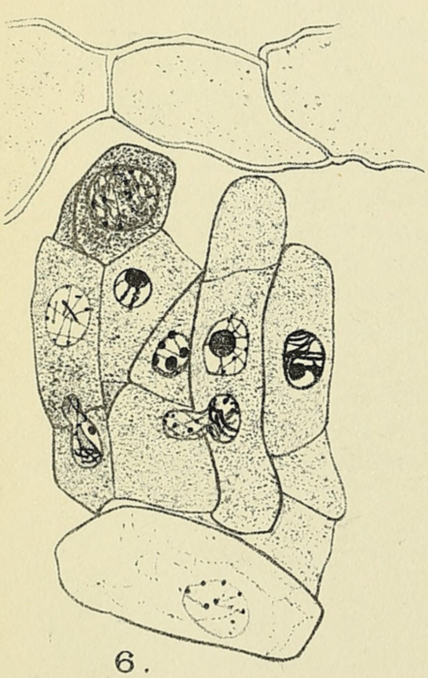

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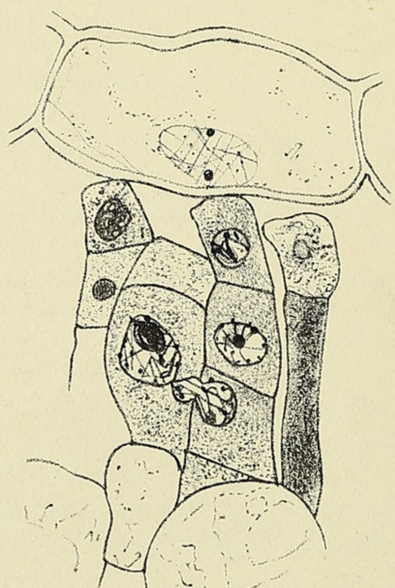

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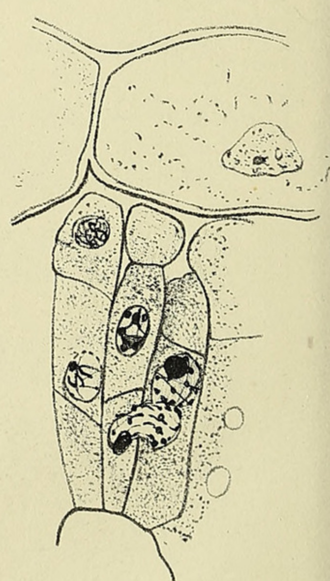

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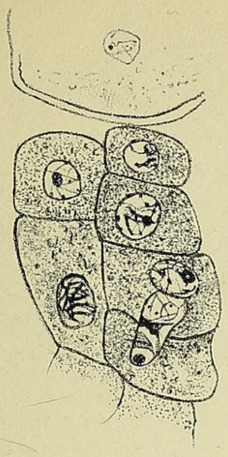
WELSFORD — PHRAGMIDIUM VIOLACEUM.



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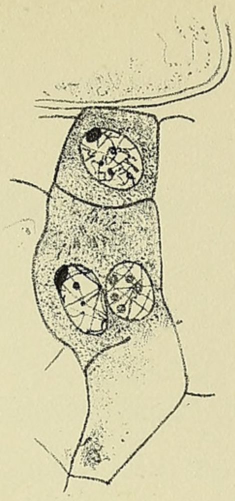

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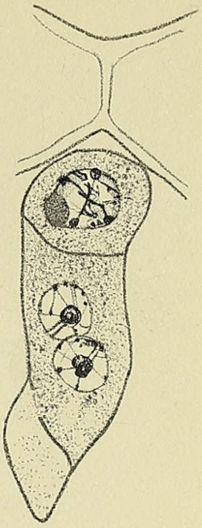

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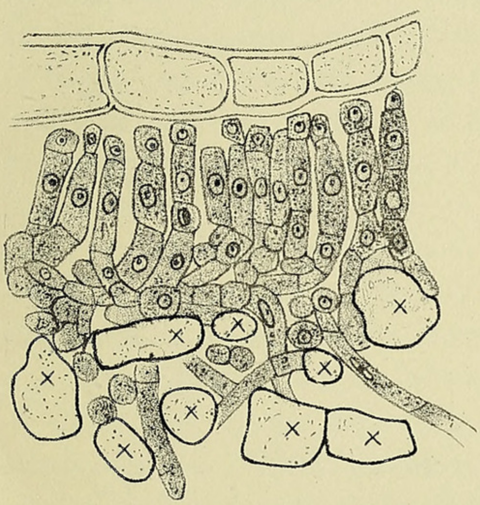

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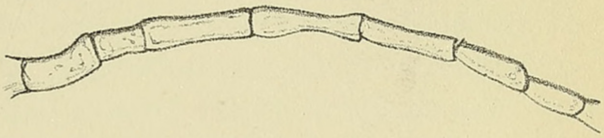

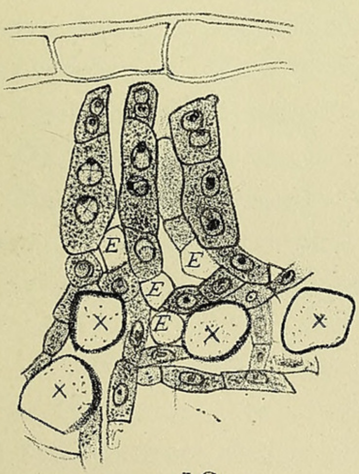

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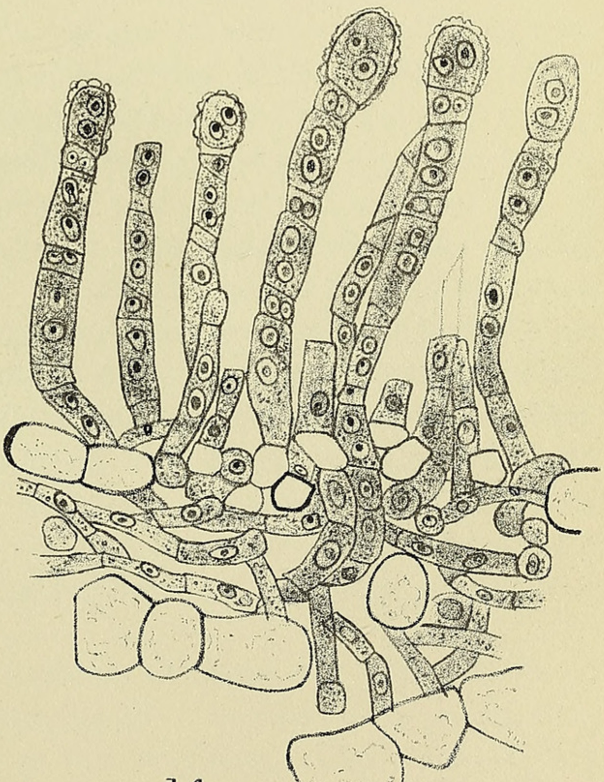

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