On the Fertilization, Alternation of Generations, and General Cytology of the Uredineae¹.

BY

VERNON H. BLACKMAN, M.A., F.L.S.,

Fellow of St. John's College, Cambridge; Assistant, Department of Botany, British Museum.

With Plates **XXI-XXIV.**

THE question of the sexuality of the Uredineae has been a vexed one ever since the suggestion put forward by Meyen (34), more than sixty years ago, that the spermogonia and aecidia represented the male and female organs. This view seemed to receive support from the later observations of Tulasne (53), and De Bary (3, 4), who showed that not only were spermogonia and aecidia closely associated in a large number of forms, but also that the spermatia produced in the spermogonia were apparently wanting in any power of germination. It appeared, then, possible that the aecidium was the result of the fertilization by a spermatium of a definite female reproductive organ. All workers, however, failed either to trace this process or even to produce any evidence of the existence of sexual organs at any stage in the development of the aecidium. These results, together with the observation that aecidia were sometimes produced in the entire absence of spermogonia (see De Bary (4); also Klebahn (27), p. 194), seemed to negative the view of any actual fertilization by the spermatium.

Owing in part to these observations and to the discovery by Cornu (12), in 1875, that the spermatia were capable of germinating to a slight degree in nutritive solutions, and also to the later observations of Möller (36) that the spermatia of some lichens could develop a mycelium under similar conditions, Brefeld (10) was led about 1889 to put forward the view that the Uredineae (and all the higher Fungi) were without any trace of sexuality and that the group should be considered as a family of the Basidiomycetes. In his view the spermogonia and spermatia, in this group and in the lichens,

¹ A short preliminary account of the chief results of this work appeared in the *New Phytologist*, vol. iii, 1904, pp. 24-27.

are nothing more than accessory asexual reproductive organs to which the terms pycnidia and conidia should more aptly be applied. These ideas were followed by Van Tieghem (54) and by Vuillemin, and have of late years gained considerable acceptance¹.

The great objection, however, to the view that the spermatia are of conidial nature is that they seem quite incapable of causing infection; the fact that they appear about the same time as the aecidiospores is also an argument against this view, as will be shown later.

Although it is clear that a careful cytological study would most likely throw great light on the vexed question of sexuality in this group, yet for a very long time our knowledge of the cell structure of the Uredineae was confined to a few scattered observations by Schmitz (49) and Rosen (45). About ten years ago, however, the researches of Poirault and Račiborski (43) and of Sapin-Trouffy (48), a pupil of Dangeard's, threw very considerable light on the cytology of this group. The former observers showed that in many stages of the Uredineae the nuclei were to be found closely associated and dividing in pairs (the *conjugate nuclei* of these authors), each pair in a separate cell. Sapin-Trouffy carried the matter further, and showed that the following very interesting cycle of nuclear development was to be observed in the forms possessing an aecidium (*Eu-* and *-opsis* forms). The mature teleutospore is always uninucleate and gives origin to four uninucleate sporidia from which a mycelium arises in which the nuclei are arranged singly, usually in separate cells. In the aecidium, borne by this mycelium, the nuclei, however, become paired; the aecidiospores thus contain two nuclei, and the paired condition of the nuclei is retained throughout ensuing mycelia and uredospores (if present) up to the teleutospores, which in the young state are binucleate, but at maturity become uninucleate by the fusion of their nuclei. In all cases of division of the paired nuclei the two are very closely associated, and a half of each nucleus goes to the new cell, so that the two nuclei in the cells produced by division are never daughter-nuclei.

These very striking observations throw no light on the nature of the spermatia, for though the latter were shown to be uninucleate, like the cells of the mycelium on which they are borne, Sapin-Trouffy assumed their conidial nature and paid little attention to them. For him the most important stage in the cycle just described was the fusion of nuclei in the teleutospore, a fusion which he considered to be of the nature of a true sexual process², and to be comparable with the fusion in the ascus and in the basidium. On the facts observed, however, there does not seem sufficient evidence for such a view; for a process of fusion which takes place in a cell

¹ A full account of the older observations on the nature of the spermatia and the sexuality of the group is given by Klebahn (27, pp. 194–202).

² Dangeard and Sapin-Trouffy (18) had earlier considered it as a 'pseudofécondation.'

which shows no signs of specialization as a female reproductive cell, and one in which the ancestors of the fusing nuclei have been associated together in the same cell for many thousands of generations, lacks all of the characters of a sexual process except that of mere nuclear fusion.

It would, in fact, seem obvious that the critical point for investigation in relation to the question of sexuality is the early development of the aecidium, for it is there, in the full life-cycle, that the two nuclei first become associated, and the transition from the single to the paired condition takes place. Sapin-Trouffy, however, imbued with the idea of the importance of the fusion in the teleutospore, paid little attention to this point, but merely states that from the mycelium with single nuclei, binucleate hyphae grow up which cut off a series of binucleate aecidiospore-mother-cells, from which by division the binucleate aecidiospores are derived.

The other results obtained by this worker, such as those on the details of nuclear division (in which he describes the regular presence of two chromosomes and the absence of a spindle) and on chromosome-reduction, left much to be desired, owing to the insufficiency of the methods employed. The figures which he gives are also of a very diagrammatic nature. In spite, however, of the obvious need of further work, the only later contributions of importance are those of Maire (29, 30), published in part after this work was in progress. Maire accepts the results of Sapin-Trouffy, but states that the cells of the aecidium which give origin to the aecidiospore-mother-cells are, in *Endophyllum Sempervivi*, De Bary, and in *Puccinia Bunii*, DC., at first uninucleate, but later become binucleate by a process which he believes to be one of simple division.

The result of cytological observations, as far as they go, is thus to suggest that the Uredineae are totally wanting in any ordinary form of sexuality or of sexual organs, and that the aecidiospores are produced by a structure of the nature of a conidiophore, in which, however, a peculiar association of nuclei takes place. The spermatia, however, still remain as very puzzling structures on which these investigations throw no light, for they are apparently completely wanting in function.

It is interesting to note that during last year Arthur (1), while admitting our ignorance of the question of sexuality in the group, has put forward the view that the aecidium is 'a device to restore vigour to the fungus,' as after this stage the parasite usually spreads and develops very rapidly; the sexual nature of the aecidium is thus suggested on physiological grounds.

An investigation for the purpose of studying the vexed question of sexuality and for settling some of the points left doubtful by Sapin-Trouffy had been projected ever since the appearance of Sapin-Trouffy's work, and was carried out during the last two years. It was clear that what was required was not so much a rapid survey of the whole group, as had been done by Sapin-Trouffy, but the careful investigation of a few

forms with special attention to aecidium-development and the character of the spermatia. With this object there were chosen as likely to be favourable objects, *Phragmidium violaceum*, Wint.¹, a common autoecious *eu*-form found on the various forms of *Rubus fruticosus*, and *Gymnosporangium clavariaeforme*, Rees, a heteroecious form with the spermogonial and aecidial stage on various species of *Crataegus*, and the teleutospore stage (uredospores are wanting) with a perennial mycelium on *Juniperus communis*, L. Material of the last-named form was obtained in abundance from the neighbourhood of Crockham Hill, Kent, where the infected Hawthorn and Juniper are often to be found in close proximity.

A careful study of these forms, especially of *Phrag. violaceum*, shows very clearly that a definite *fertilization* is to be observed in the aecidium, that the Uredineae show a well-marked alternation of sexual and asexual generations, and that the fusion in the teleutospore, the nature of which has been so much disputed, is relatively of unimportance, being a mere preliminary to reduction.

METHODS.

The material used was fixed chiefly with either Flemming's fluid (usually the weak formula) or with acetic alcohol which was allowed to act only for a short time. All material, except that for the study of teleutospore-germination, was fixed in the field, an air-pump being used in the case of the non-alcoholic fluids to remove air from the surface of the pieces and so facilitate the penetration of the fluid. The material after fixing, washing, and dehydrating was preserved in equal parts of alcohol, glycerin, and water. For staining, Flemming's triple stain, Benda's iron-haematoxylin, and brazilin were all found very useful; for the latter stain it was found convenient to use, instead of the ordinary alcoholic iron-alum solution, which goes bad very quickly, a solution made by diluting Benda's '*Liquor ferri*' with about nine parts of 70% alcohol. This mixture remains unprecipitated for many months.

For the study of teleutospore-germination *G. clavariaeforme* was found very favourable. The masses of teleutospores will retain their power of germination in a cool place for over a month. If pieces of the bark with the masses attached are placed for a few seconds in water under an air-pump so as to get them thoroughly wet, and then in Petri-dishes over damp filter-paper at a temperature of 20° C., germination takes place very readily and sporidia are formed in less than three hours. Very good results in the study of nuclear divisions in the germ-tube were obtained by the use

¹ The characters which distinguish this species from that of *Phrag. Rubi*, Wint., are very unsatisfactory and the two forms should probably be united. The form here investigated is obviously the same as that studied by Sapin-Trouffy, which he calls *P. Rubi*, but as there are usually four cells to the teleutospore and only a slight projection at the apex the other name seems preferable.

of Flemming's weaker fluid diluted with an equal quantity of water. After fixing and washing, the material was brought up to the clearing fluid by the method of Overton (41). It was first placed in 10% glycerin, which was allowed to evaporate either in a warm place or in a desiccator over calcium chloride; the glycerin was then removed with absolute alcohol and the material placed in a 10% solution of cedar oil in alcohol. After the alcohol had evaporated in a desiccator the material was obtained in cedar-wood oil without the least distortion of the delicate germ-tubes, and with far less trouble and risk than by the passage through various strengths of alcohol, and mixtures of alcohol and clearing fluid. The material can then either be treated by the method of fixing to the slide (see Blackman, 8), or by imbedding small teased pieces in hard paraffin very successful sections can then be made through the teleutospores and germ-tubes. By the latter method alone can the details of nuclear division be successfully studied. For staining the cell-walls of the hyphae a 1% watery solution of Congo-red, either neutral or slightly alkaline, was found of great use, as it stains the walls of the hyphae without affecting the host-cells.

I have to thank Miss H. C. I. Fraser for very considerable help in preparing material during the latter part of this work.

TELEUTOSPORE AND PROMYCELIUM.

PHRAGMIDIUM VIOLACEUM.

The peculiar nuclear cycle with its sudden transition in the aecidium, as described by Sapin-Trouffy, was fully confirmed in the case of the two forms investigated. It will thus be convenient to start with the first stage of the series with single nuclei—the mature teleutospore—and then work by way of the spermogonia and aecidia to the series with paired nuclei, and after dealing with the uredospores end with the development of the teleutospores and the fusion of the two nuclei into one.

Mature Teleutospore. The teleutospore of this form appears towards autumn, and is to be found in groups on the leaves which remain attached throughout the winter. It is a large structure consisting usually of four thick-walled cells, and a stalk which is enlarged below, this part of the stalk being capable of swelling up in water to a great extent (Fig. 1). The wall of the uppermost cell is provided with a rounded peg-like projection of cell-wall substance which is sometimes perforated by a narrow canal connected with the cavity of the cell below. The whole spore is covered with a thin cuticular layer which is raised into numerous rounded bosses (Figs. 1 and 2). Each cell possesses a delicate endosporium, a continuous, thick mesosporium, and an outer exosporium, which is to be found only as

a sheath round the two middle cells, but exists also round the upper part of the top cell and the base of the lowest cell (Fig. 2). The stalk has a narrow lumen which widens out below (Figs. 5 and 86 *c*); its wall shows only slight differentiation of layers. Each cell has usually four pores which are closed on the outside by the cuticle only, the protoplasm and endosporium projecting slightly into the cavity in the mesosporium and exosporium (Fig. 2). Each cell contains a single central nucleus with a well-marked nucleolus, and dense cytoplasm containing a quantity of yellow oily material which gives to it an orange colour. In the dry state very little can be made of the structure of the nucleus, and except for the nucleolus it appears almost homogeneous.

Germination. Spores taken in March from the 'wintered' leaves germinate readily in moist air or water, but will not do so earlier. The first beginning of the germ-tube (promycelium) is the welling out through one of the pores of a spherical mass of yellow cytoplasm surrounded, of course, with a thin wall. This stage (Fig. 1) is very striking, and is also to be observed in *G. clavariaeforme*. From this spherical mass the cylindrical germ-tube grows out away from the spore (Fig. 5).

The nuclei undergo an interesting series of changes when the spores have been lying in water some time. After twelve hours in this condition the nucleus increases in size, and there is to be observed a somewhat flattened nucleolus pressed close against the nuclear membrane, which often projects at this point. The faintly staining, and hitherto almost homogeneous, mass of the nucleus begins to stain more darkly, and faint indications of a chromatin thread are to be seen (Fig. 3). Soon the chromatin part becomes clear as a single much-twisted thread, at first closely coiled; but as increase in size of the nucleus still goes on the coil becomes a more open and thinner spireme thread. This stage, however, is not a preparation for division, for it appears to last only a short time, and very soon the nuclei return to the ordinary condition and show a nucleus with a chromatin network, and, instead of a single large nucleolus, a few smaller ones (Fig. 4). The nucleus has a large cavity and is only partly filled with the network, so that in the living teleutospore examined in water before germination it appears as a distinct, clear 'vacuole' (Fig. 5), as it was termed by the older observers.

In some few cases *two* smaller nuclei are to be found in the cell of the teleutospore (Fig. 4 *a*) instead of one larger one. These are, no doubt, the original paired nuclei of the teleutospore, which for some reason have delayed their fusion. Their fate is unknown, as two nuclei were never observed to pass into the germ-tube; probably they fuse later. There was no evidence that they represented the results of a precocious division.

The nucleus passes into the germ-tube, being constricted in its passage through the pore, which is only of small diameter (Fig. 6). If the germ-tube

does not reach the air, it usually grows on continually (Blackman, 7), the protoplasm with the nucleus being found at the end of the tube (Fig. 7).

Division of nucleus. This form is not a favourable object for the study of this process on account of the irregularity of the germination and the difficulty of cutting the necessary sections. The divisions in the promycelium were studied fully in the form to be next described, but here it was merely observed that not two (as Sapin-Trouffy states), but numerous chromosomes were formed on division, and that there was a definite spindle with centrosomes.

Sporidia-formation. The four cells of the promycelium contain only small nuclei with a very small amount of chromatin. The sterigmata produced may either be short, as in the typical condition seen in Fig. 8a; or elongated, with more of the appearance of germ-tubes (Fig. 8). The difference appears to depend upon the degree of moisture present (vide infra under *Gymnosporangium*). The whole of the protoplasm does not pass into the sporidia, but a small quantity remains in the promycelial cell (Figs. 8a and 9). The sporidium is a globular or pear-shaped structure with a wall of some slight thickness and irregular in outline (Fig. 8a), a nucleus which sometimes shows no nucleolus, but a well-marked chromatin network (Fig. 10).

The sporidia germinate very readily, sometimes while still attached to the sterigma (Fig. 9), and often form a secondary sporidium which seems always to be binucleate (Fig. 11), and in one case four nuclei were apparently to be observed (Fig. 13). This binucleate condition is also sometimes to be observed in the primary sporidium even while it is still attached to the sterigma (Fig. 12). The condition with two nuclei appears to be fairly common in the primary or secondary sporidia of the group. It has been described in *Puccinia Malvacearum* by Sapin-Trouffy (48), in *Endophyllum Sempervivi* by Maire (29), and for *Coleosporium Euphrasiae* by Poirault and Račiborski (43), and it is to be sometimes found in *G. clavariaeforme*. It appears to be entirely without significance and to be merely a precocious division of the nucleus in which the usual wall-formation is delayed. The nuclei are certainly not paired (conjugate), for in *Phragmidium* and the first two species mentioned above the sporidia have been observed to give rise to a mycelium with single nuclei as in all other known cases; in fact in *E. Sempervivi* Maire states that the two nuclei can be observed to pass into the infecting germ-tube and there become separated by a septum.

GYMNOSPORANGIUM CLAVARIAEFORME.

Mature Teleutospore. The two-celled teleutospores of this form, with their very long gelatinous stalks by means of which the spores are held together in compact yellow masses, are well known (Fig. 14). As noticed by

earlier observers they are of two kinds, thick-walled and thin-walled, the latter being found more usually in the interior of the mass; it is possible, also, that there may be some connexion between the thickness of the wall and the time of year at which the spores are formed, for the first-formed spores seem to be almost all thick-walled¹.

Each cell of the teleutospore contains dense vacuolate cytoplasm with a quantity of yellow oily material which gives the colour to the spore. There is a single nucleus of considerable size with a chromatin network arranged superficially round the cavity and one or more small nucleoli (Figs. 14 and 15). The wall of the spores shows usually no clear distinction into layers. Near the septum, the wall of each cell (of the thick-walled spores) is thinner at one spot, and into the cavity so produced the protoplasm projects slightly (Fig. 14). It would seem that the wall is never thickened at these spots, for such thin places in the wall are found in comparatively young spores; they are thus to be considered of the nature of pores. These pits are not to be found in the thin-walled spores (where they are obviously unnecessary), but the naturally thin wall may project slightly at the corresponding points.

Germination. Under suitable conditions of moisture and temperature germination takes place very readily, in a moist atmosphere at 20° C. the promycelium being formed and the sporidia developed in less than three hours. The cytoplasm forces its way through the pore described above, and, after swelling out into a spherical mass, as in *Phragmidium*, attains a considerable length before the nucleus migrates into it (Fig. 16). The nucleus, very much constricted and condensed in its passage through the pore, takes up a position in the middle of the tube and is there to be seen as a narrow elongated body with a few small nucleoli, and granular chromatin which forms usually only an indistinct network. Under suitable conditions of air-supply the nucleus then proceeds to divide.

Nuclear division. The first sign of division is the gathering of the granular chromatin material towards one end or the middle of the nucleus, while at the same time a small, deeply staining, spherical body, the centrosome, is to be observed in the cytoplasm and connected with the nucleus by a portion of slightly differentiated cytoplasm or kinoplasm (Fig. 17). The chromatin then forms a closely coiled spireme thread in the middle of the nuclear area and the outline of the nucleus becomes very faint (Fig. 18); the centrosome was not traced at this stage. The thread then becomes apparently broken up into a number of narrow, elongated

¹ The suggestion put forward by Kienitz-Gerloff (Bot. Zeit., xlvj, 1888, p. 388) and Dietel (Hedwigia, xxviii, 1889, p. 99), that the thin-walled spores represent in this genus the endospores, cannot be accepted. Both kinds of spores are uninucleate, and both are able to form normal sporidia; the long, undivided tubes put out by the thin-walled spores were no doubt the result of special conditions of growth (see Blackman, 7).

chromosomes (Fig. 19 *a*), but they lie so close together that their exact number and length are difficult to make out; there are certainly at least ten. At this stage the spindle was first observed as a very short structure with well-marked centrosomes at each pole, lying among, but without any distinct relation to, the chromosomes (Fig. 19 *b*). From the fact that the centrosome with a mass of kinoplasm was first observed outside the nucleus, and from analogy with the second division, there can be no doubt that the spindle is formed in the cytoplasm, between two centrosomes which arise by division of the one observed earlier, and that it later comes into association with the chromosomes.

The spindle gradually increases in length, and the closely packed, ill-defined chromosomes come into close relation with it (Fig. 20). As the spindle lengthens the chromosomes become confined to the more central part of the spindle (Figs. 21, 22), which, if originally oblique (Fig. 22), takes up a position parallel to the long axis of the germ-tube. The spindle still continues to elongate and the chromosomes to spread out, so that when the spindle has attained its full length, they occupy usually two-thirds of its length (Figs. 23-5).

This stage, at which the spindle has reached its full length, seems to correspond with the metaphase, being the one most frequently met with; but no distinct equatorial plate is ever formed. At this stage the form of the chromosomes is usually almost completely lost, and they appear to have partly fused together, a few free ends (Fig. 23) or the presence of irregular lumps of chromatin (Figs. 24, 25) alone suggesting their existence.

The chromatin material then begins to move towards the poles, but rarely, as in Fig. 26, are two distinct polar groups to be seen. Usually this stage cannot be sharply distinguished from the last, and the chromatin lumps merely become strung out from pole to pole and often showing a tendency to form two elongated masses (Fig. 29). Sometimes two irregular twisted threads forming two clumps can be seen moving towards each pole (Fig. 28), and occasionally the masses moving towards the poles can be seen to have formed two chromatin networks (Fig. 27). After this stage, the suggestion of separate chromosomes becomes usually completely lost and the chromatin merely stretches out from pole to pole as two elongated dumb-bell-shaped masses (Fig. 30). The chromatin then collects at the poles, forming there one irregular mass (Fig. 31), or the distinction into separate masses may be clearly maintained at each pole (Fig. 32).

During these stages the centrosomes show clear but faintly staining polar radiations, which are often of very considerable length (Fig. 24), and in the case shown in Fig. 31 extended to the free end of the germ-tube. When the chromatin has collected at the poles the two (or four) masses move apart

and the spindle becomes stretched out and very narrow; the centrosomes and radiations are however still clearly visible (Fig. 31). The daughter-nuclei are then formed; each is a somewhat pear-shaped body and consists of a mass of perfectly homogeneous staining material. At the pointed end of each, the centrosome can be clearly observed; it is not attached directly to the nucleus, but is connected with it by means of a small portion of kinoplasmic material which seems to correspond with the end of the former spindle (Fig. 33). The centrosome shows distinct radiations (Fig. 33).

Under the conditions under which the teleutospores were germinated the two second divisions follow immediately on the first. The centrosome becomes divided into two (Fig. 34), and between them the spindle appears (Fig. 34 *a*). At the same stage the nucleus becomes larger and irregular in shape, and a few granules appear in it; in Fig. 34 *a* they are to be seen just where the spindle is in contact with the nucleus, over the edge of which it lies. The spindle then becomes arranged in the long axis of the germ-tube, and shows distinct polar radiations, while the nucleus, which has shown no definite wall from its first formation, is seen as a mass of chromatin lying in an irregular way over the spindle (Fig. 35). The chromatin then becomes more symmetrically arranged round the spindle, and is seen to be granular throughout (Figs. 36, 37). The chromatin then takes the form of a distinct *network*, which at first covers only part of the spindle (Fig. 38 *a*), but soon spreads over the whole of it, leaving only the centrosomes visible (Fig. 38 *b*). The network of chromatin then becomes drawn apart as two portions towards the poles (Fig. 39). In a later stage the threads which connected the two main masses may remain visible for a time at the poles, and resemble chromosomes (Fig. 40). The chromatin then collects at each pole usually as a single mass (Fig. 41), but not infrequently it forms two distinct masses (Fig. 41 *a*). The four daughter-nuclei when they are first formed show, as in Fig. 42, a somewhat pear-shaped mass of lightly staining homogeneous material, which bears at the pointed end a mass of kinoplasm and a distinct centrosome, as in the corresponding stage of the first division. The promycelium then becomes divided into four cells, and the nuclei gradually take on a normal appearance, a chromatin network becoming visible and the centrosome apparently disappearing (Fig. 43).

It is clear that though the division in the promycelium of *Gymnosporangium* is much more typical than Sapin-Trouffy supposed, yet there is certainly no chromosome-formation in the second division, and in the first division, though distinct chromosomes appear to be present, they seem soon to lose their individuality, so that of their splitting or regular separation there is very considerable doubt.

The two chromatin-groups, which are often to be observed both in

the first and second division, probably represent the chromatin derived respectively from the two nuclei which fused in the teleutospore. It was these two masses which Sapin-Trouffy mistook for two chromosomes, as a comparison of his figures with Figs. 30, 32, and 41 *a* clearly show.

The method of spindle-formation, in which the spindle is formed free in the cytoplasm between two centrosomes and later comes into relation with the dividing nucleus, is of great interest. It is clearly of the type described, in animals, by Hermann and others for the *Centralspindel*. The only case in plants with which it is at all comparable is the peculiar method of spindle-development described by Lauterborn (28) for Diatoms¹.

Since the work of Sapin-Trouffy, who failed to discover either a spindle or chromosomes in the divisions in the promycelium, it has been shown by Juel (25), in *Coleosporium Euphrasiae*, that a spindle and polar radiations were present, and that the division was of a much more typical nature than Sapin-Trouffy had described. He was not, however, able to determine the behaviour of the chromatin part of the nucleus. When this work was complete a paper appeared by Holden and Harper (22), in which it was shown that in the divisions of the developing teleutospore of a form of *Coleosporium* on *Callistephus*, &c., to which they give the name *C. Sonchiarvensis*, a spindle with centrosomes, polar radiations, and showing numerous chromosomes was to be observed, as in *Gymnosporangium*. They did not, however, trace the origin of the spindle, and they give but a few figures of the behaviour of the chromatin, for *Coleosporium* does not seem to be nearly so favourable an object of study as the form here investigated. They are of the opinion that both divisions are typical in nature.

Sporidia-formation. There is nothing worthy of special comment in the normal formation of sporidia. A primary sporidium is shown, in section, at Fig. 25.

In some cases the original four cells of the promycelium may round themselves off, separate, and put out germ-tubes, thus behaving like sporidia. Long germ-tubes may also be put out by the four cells while they are still attached, as shown in Fig. 44. Although the exact conditions were not investigated, this method of shortened development seems to be dependent on conditions of moisture, and is probably a response to growth under water or at least in a very moist atmosphere. It has been shown in an earlier paper (7) that promycelia actually growing under water develop to a great length, but rarely divide, and never form sporidia. A single case was there figured for *Phragmidium*, where the promycelium had divided

¹ P. Denke (Beihefte z. Bot. Centralbl., xiii, 1902, p. 182) has described the development of an extra-nuclear spindle, though, of course, without centrosomes, in the division of the microspore and megaspore mother-cell of *Selaginella*.

under water, and one of the four cells was putting out a germ-tube¹. Inside the gelatinous mass of teleutospores access to free air must be difficult, and it is probably in such a situation that the promycelial cells tend to germinate directly.

Such cases as these of *Gymnosporangium* and *Phragmidium* show that the real germinating unit is the promycelial cell, and that under certain conditions it can become a separate spore, put out a germ-tube and behave as a sporidium, and no doubt cause infection. When growing normally, however, in air the development of the germ-tube is arrested, and it becomes a mere sterigma, on the end of which the primary sporidium is developed. The primary sporidium thus appears to be merely the arrested and swollen germ-tube put out by the promycelial cell, just as the secondary sporidium is merely the arrested and swollen germ-tube of the primary sporidium. The sporidia are probably nothing more than special adaptations to the development of the promycelium in air; in the absence of air such a development would be unnecessary, for there would be no chance of the wind-dispersal of the sporidia. The primary sporidium is thus really secondary in nature, and bears the same relation to the promycelial cell (the true primary spore) as does the so-called secondary sporidium to the first-formed one.

It is evident that the formation of the so-called promycelial cells is really nothing more than the division of the contents of the teleutospore into four spores, which may separate as such, but usually remain united and form four secondary spores—the sporidia. As far as is known, the teleutospore is quite incapable of forming a mycelium; it seems then time that the term *promycelium* should be dropped, and the process of development considered merely as one of spore-formation.

That the later development of the teleutospore is really a process of spore-formation has been obscured not only by the formation of sporidia (really the secondary spores), but also by the fact that, owing to the thickness of the spore-wall, a process of germination is a necessary preliminary to division. In *Coleosporium*, however, where the teleutospores are thin-walled and develop *in situ*, there is no process of germination, but the spores become directly divided into four cells, which normally put out each a sterigma and form sporidia. Holden and Harper (23), however, in this genus have lately observed cases in which the divisions of the teleutospore rounded themselves off and became directly spores. In such cases, the teleutospore, its real nature no longer obscured either by the process of germination or by the formation of sporidia, is clearly seen to be a *spore-mother-cell, which undergoes a tetrad division to form four spores*.

¹ A somewhat different interpretation was placed upon this case at the time, but a comparison with *Gymnosporangium* gives the clue to its real nature. Sapin-Trouffy (48) has described a case in *P. Malvacearum* in which submerged promycelial cells separate and put out germ-tubes, which become sterigmata (and bear sporidia) only if they reach the air.

MYCELIUM AND SPERMOGONIA.

PHRAGMIDIUM VIOLACEUM.

The mycelium which appears in the leaf soon after germination of the teleutospores is derived by infection from the sporidium, and soon bears the spermogonia; it consists of numerous hyphae with *single* nuclei, which appear to be always enclosed in separate cells. Most of the hyphae are intercellular; some penetrate the cells, but they appear to have no definite connexion with the nuclei of the host-cells, as in the haustoria described by Sapin-Trouffy for some forms.

The spermogonia are found usually on the upper side only of the leaf. Like the aecidia they are of indefinite extent, and are to be found as simple layers of parallel spermatial hyphae developed beneath the cuticle. Each irregular layer or spermogonium can be distinguished into a number of slightly projecting 'hummocks,' above which the cuticle is perforated to form the ostiole. One of these 'hummocks' is shown in section in Fig. 46. The spermogonia have usually no paraphyses, but occasionally one or two spermatial hyphae may grow out as sterile threads and project through the aperture in the cuticle.

The spermogonia are formed by hyphae which grow up between the epidermal cells, and form beneath the cuticle a mycelial bed, or plectenchyme, of uninucleate cells. From this there arises a compact mass of parallel spermatial hyphae. The spermatial hyphae of any given group all point their free ends towards the aperture in the cuticle in the centre of the hummock (Fig. 46). Each group of spermatial hyphae with its ostiole should probably be looked upon as a single spermogonium, several spermogonia being collected together to form a composite structure. The cuticle, which is of course pushed up by the growth of the hyphae, becomes very much thickened at these spots, and stains very deeply with the safranin of Flemming's triple stain and with iron-haematoxylin (Fig. 46).

Each spermatial hypha contains a single, usually elongated, nucleus with a chromatin network, one or two small nucleoli, and an indistinct membrane. The spermatia are budded off from the tips of the hyphae, a projection being first pushed out from the apex of the hypha, and when it has reached its full size the nucleus passes into it. The end of the hypha has a ring of special cell-wall, staining deeply with Congo-red, as in *Gymnosporangium*, which offers a much more favourable object for studying the development of the spermatia.

The mature spermatia are minute uninucleate cells more or less oval in shape. Each contains a comparatively very large nucleus, which shows a dense chromatin network but no nucleolus; surrounding this is a thin

layer of very finely granular cytoplasm with apparently no reserve-material, and the whole is enclosed in a very thin cell-wall.

The spermatia make their escape through the ostiole, and are found spread out on the leaf in the immediate neighbourhood. They took no part in aecidium-formation or in any other process, and numbers of them when observed on the leaf-surface appear to be in a disorganized state, in which the distinction between nucleus and cytoplasm is almost completely lost.

GYMNOSPORANGIUM CLAVARIAEFORME.

The mycelium in the leaves of the Hawthorn which bears the spermogonia shows clearly the *single* nuclei apparently always enclosed in separate cells. They are of small size, and usually show no distinct nucleolus, and their nuclear membrane is often indistinct. The spermogonia appear very early after infection, in about seven to ten days.

The spermogonia are flask-shaped structures of the usual type, and are developed beneath the epidermis. They arise from a layer of small-celled 'tissue' (plectenchyme), which gives origin to a number of upwardly directed and parallel hyphae, the spermatial hyphae. Certain of these hyphae, chiefly the peripheral ones, grow out to form the paraphyses which project through the ruptured epidermis. The others bud off each a series of spermatia, which collect in the cavity of the flask, and later become extruded (Fig. 48).

This form is a favourable object for studying the exact development of the spermatia. The spermatial hyphae are narrow, elongated cells with a central elongated nucleus, which shows a granular chromatin network and one or more small nucleoli; the nuclear membrane, however, is indistinct (Fig. 49). The hypha contains only finely granular cytoplasm. It is furnished at the free end with a curious ring of thickening, which is easily rendered visible by the fact that it takes the Congo-red with more avidity than the rest of the cell-wall (Figs. 49-54).

The first beginning of the development of a spermatium is the pushing out of a finger-like projection from the free end of the hypha, thus displacing the last-formed spermatium (Fig. 49). This projection contains exceedingly finely granular protoplasm. When it has attained its full size, or a little earlier, the nucleus of the spermatial hypha begins to undergo a process of condensation (Fig. 50), so that it is transformed into a homogeneous and deeply staining chromatin mass (Figs. 51 and 55), the nucleolus or nucleoli being apparently squeezed out into the general cytoplasm (Fig. 55 *a*). The condensation of the nucleus may begin at one end and progress downward, as shown in Fig. 50. The mass of chromatin then becomes drawn apart into two masses (Figs. 52, 56), which remain

connected by a thread of kinoplasmic substance. The two become finally separated, and the upper one, passing through the thickened girdle, moves into the spermatium (Fig. 53). The lower chromatin mass passes back into the resting state (Fig. 54), and the process is again repeated. The spermatium becomes cut off from the hypha by a wall formed just above the thickening ring, which may be connected with the disjunction of the spermatium.

As in the divisions to be described later, the nuclei sometimes show not a single chromatin mass, but *two* masses, which are drawn out separately towards the respective poles (Fig. 57).

The only trace of spindle-formation was the connecting strand between the two separated chromatin masses. It may be that a rudimentary spindle is present as in the case of the conjugate divisions, but is obscured by the chromatin mass. The whole method of division is obviously of an exceedingly simple type.

When the spermatium separates from the spermatial hypha its nucleus is an almost homogeneous and deeply staining mass (Fig. 58), but in the mature state becomes larger and shows a dense network without a nucleolus. As in *Phrag. violaceum*, the mature spermatia (Fig. 59) are small cells with a large, dense nucleus, very little cytoplasm, a thin cell-wall and apparently no reserve-material.

The spermatia, when extruded from the spermogonium, collected on the leaf in apparently sticky masses. Many of them seemed, as in *Phragmidium*, soon to undergo a process of degeneration, in which the staining distinction between nucleus and cytoplasm became lost, and in one case this process of disorganization was observed in some of the spermatia while they were still enclosed in the spermogonium.

The spermatia were never observed germinating or taking any part in aecidium-formation.

The formation of spermatia has been investigated by Sapin-Trouffy (48) in *Uromyces Erythroni*, DC., and other forms, and in *Puccinia Liliacearum*, Duby, by Poirault and Račiborski (43), and also by Maire (29, 30); the observations here given confirm and supplement their results. Both Sapin-Trouffy and Maire, however, describe two chromatin masses as always present on nuclear division, and consider them to be of the nature of chromosomes. These two structures are not always present in *G. clavariaeforme*, and they certainly cannot be considered of the nature of true chromosomes, as a comparison with the divisions in the promycelium shows, for these chromosomes are formed and are much more numerous. This matter will be discussed later in dealing with conjugate division.

AECIDIUM-DEVELOPMENT.

PHRAGMIDIUM VIOLACEUM.

The so-called aecidium in this genus, like that of *Caeoma* (= *Melemposora* in many cases), is characterized by a very simple structure, for unlike that of the other genera of the group it is neither definite in shape nor bounded by a thick-walled pseudoperidium. In the form investigated the aecidium is nothing more than a group, indefinite in extent, of aecidiospore-bearing cells, bounded at the periphery by a number of thin-walled paraphyses, which are, however, sometimes wanting. It is clear that this aecidium is no more a definite organ than is the sorus of uredospores or teleutospores, which has an exactly similar arrangement—a group of spore-bearing cells surrounded by paraphyses. The aecidiospores are developed immediately beneath the epidermis, and not deeper down in the tissues as in the typical aecidium.

The first beginning of the aecidium is the massing of hyphae beneath the epidermal cells of the leaf, usually on the lower side. The hyphae form there a layer of uninucleate cells, two or three cells thick. The cells immediately beneath the epidermis increase somewhat in size, and soon become divided by a transverse wall, parallel with the surface of the leaf, into an upper and lower cell, each with a single nucleus. The upper cell remains more or less cubical, and shows a vacuolate protoplasm and a small nucleus, which has no nucleolus and sometimes remains as a dense structure without returning completely to the resting state (Fig. 66). The lower cell elongates considerably, and shows abundant granular protoplasm and a large nucleus with a well-marked nucleolus and a clear chromatin network (Figs. 61, 66, 70). The upper cell is a *sterile cell*; its nucleus becomes disorganized, and it is soon destroyed by the upward growth of the cell below. It is from the lower cell, which may be called the *fertile cell*, that the aecidiospores arise; for after a pause in its development it becomes *binucleate*, and proceeds to elongate (Fig. 61) and cut off a series of binucleate aecidiospore-mother-cells (Fig. 60), the pair of nuclei dividing together by the process of *conjugate division*.

The discovery of a sterile cell is alone a very important fact, for it shows that the aecidiospore-producing cell or hypha, at least in *Phrag. violaceum*, is a very specialized reproductive cell and not simply of the nature of an ordinary conidiophore.

The process by which the fertile cell became binucleate was naturally closely investigated, but all attempts to observe the single nucleus of the fertile cell in any stage of division were quite unavailing, in spite of the fact that other divisions were met with fairly frequently, and that in passing from the periphery to the centre of an aecidium of an appropriate

age one can observe the transition from the uninucleate to the binucleate condition. This failure led to a close examination of the fertile cells at about the point of transition, and it was observed that in a number of cases the fertile cell was occupied by two nuclei of different size and structure (Figs. 62-65). One was usually a larger nucleus with the characters given above for the original nucleus of the cell, the other, usually a smaller, denser nucleus containing no nucleolus or only a small one, and having, in fact, more the characters of a nucleus of an ordinary cell of the mycelium.

The differences in size and structure of these two nuclei was hardly compatible with the view that they were sister-nuclei, and when this fact was further considered in conjunction with the absence of all stages of the necessary division, there seemed no escape from the view that the smaller, denser nucleus must have had some other origin. This view was amply confirmed by the discovery of a number of cases in which *a nucleus was actually found passing into the fertile cell from one of the smaller cells of the mycelium at its base* (Figs. 66-70)¹. The migrating nucleus is reduced to a narrow thread during the process, the actual aperture through which it passes being very small; neither before nor after its passage could a pit in the wall be observed².

In many cases there is to be found lying below the fertile cell a cell which obviously belongs to the same cell-row; it may be called a *basal cell*, though it is in no way specially differentiated. When this basal cell is present it is often from it that the migrating nucleus comes; and it is interesting to note that the nucleus seems to pass more often into one of the neighbouring fertile cells (Figs. 66, 67, and 71), with which it is in contact at the sides owing to the irregularity in length of these cells, rather than into the fertile cell immediately above it (Figs. 68 and 69). In the former case the relationship between the two nuclei which meet in the fertile cell may be considerably distant, while in the latter case the two nuclei are separated in origin only by one division, that which cuts off the sterile cell.

In some cases in which the fertile cell contained two dissimilar nuclei, the smaller, denser one lay in the upper part of the cell, and at the same time the sterile cell was without a nucleus (Fig. 65). It seemed then possible that the nucleus of the sterile cell might have migrated into the fertile cell below; but no direct evidence could be obtained for this view, and it is rendered improbable by the fact that a similar state of affairs was

¹ More than twenty-four cases were observed in which the fertile cell contained two dissimilar nuclei and more than fifteen cases in which a nucleus was in a stage of actual migration.

² The migration of nuclei in the tissues of Phanerogams which have been placed under abnormal conditions has been described by several observers (cf. Koernicke, Ber. d. Deutsch. Bot. Ges. xxi, 1904, p. 100).

to be observed in the fertile cell while the nucleus of the sterile cell above was still in position (Figs. 63 and 64). The disappearance of the nucleus of the sterile cell is doubtless to be accounted for by early disorganization, and the peculiar position in the fertile cell of the small nucleus by a change in position after migration, or by the fact of its having entered at the side.

It would appear that the two nuclei of the fertile cell soon become similar in size and shape, for it is only occasionally that a dissimilarity can be observed. Very soon after this condition has been attained the fertile cell proceeds to elongate, pushing its way through the sterile cell (Fig. 61) and soon completely destroying it. The paired nuclei divide by the process which has been termed conjugate division, and the fertile cell cuts off a series of cells (aecidiospore-mother-cells), which do not develop directly into aecidiospores, but each cuts off a small cell below, known as the intercalary cell (Figs. 71-74). It has been suggested that the function of this cell, which becomes disorganized and disappears soon after its formation, is to act as a disjunctive apparatus, and so bring about the complete separation of the aecidiospores. A mature aecidiospore is shown in Fig. 75. Most of the fertile cells become binucleate and develop further, for in older stages of the aecidium only a few of the fertile cells are found, here and there, still uninucleate. It is no doubt these cells, which never achieve the binucleate condition, that are found crushed and distorted in still later stages¹.

Although two is the usual number of nuclei in the fertile cell, the number *three* is nearly always to be found in one or two cells of each aecidium, and in one case it was found no less than four times in a single aecidium; but in comparison with the normal number it is of course very uncommon. The three nuclei divide together by a process of conjugate division (Fig. 77), and trinucleate aecidiospore-mother-cells (Fig. 76) and aecidiospores are produced, the fate of which is unknown. In one case also a fertile cell with four nuclei was to be observed.

How this multinucleate condition is brought about is not quite clear; it may be due to the migration of more than one nucleus into the cell, or the division of one or both of the nuclei without cell-wall formation. In a fertile cell which was yet undivided one of the nuclei was observed to be apparently undergoing division while the other was in the resting state. When three or four nuclei were observed in an undivided fertile cell it was interesting to note that their size was usually considerably less than that of the normal paired nuclei.

¹ The fertile cells are often in contact with several of the cells of the tissue below, owing to the irregularity in length of the former, and the usually narrow form of the latter when they do not form distinct *basal cells*; a more than sufficient number of nuclei is thus at hand to supply all the *fertile cells*.

The development of the aecidium is centrifugal, and when it has reached a certain size the peripheral cells, instead of forming ordinary sterile and fertile cells, grow out into uninucleate paraphyses: these may, however, in some cases may be absent.

The process of nuclear migration, by means of which the fertile cell is stimulated to rapid growth and continued division, is clearly a process of *fertilization*, the exact relation of which will be discussed later¹.

GYMNOSPORANGIUM CLAVARIAEFORME.

The first beginning of the aecidium takes place deep down in the hypertrophied tissue of the leaf, &c., after the majority of the spermogonia have faded. The aecidium in this genus is of the typical form, with a definite pseudoperidium. Unfortunately the very early stages, which are much more difficult to obtain than in *Phragmidium*, were not observed, so that the presence or absence of sterile cells and the exact behaviour of the nuclei could not be investigated. It was clear, however, that the transition from the condition of single to that of paired nuclei took place, as described by Sapin-Trouffy, in connexion with the aecidiospore-bearing cells (fertile cells); for while in the whole mycelium, and even in the dense layer of fungal cells surrounding the actual aecidium, the nuclei were single, yet the fertile cells and their products showed clearly two nuclei in the paired (conjugate) condition.

It is evident that a comparative study of aecidium-development in the Uredineae generally, with special relation to the existence of sterile cells and the origin of the binucleate condition, is much to be desired. The only other forms of which we have any information are *Endophyllum Sempervivi*, De Bary, and *Puccinia Bunii*, DC., in which Maire (29, 30) is of opinion that the two nuclei of the fertile cell are daughter-nuclei arising by division of the original single nucleus. The migration of nuclei, however, is very easily overlooked unless special attention is drawn to such a point; it is interesting to note that Maire does not appear to have observed in *Puccinia Bunii* the actual division, for he merely states, 'A little later, it can be seen that the terminal cells [fertile cells] contain each two nuclei' (30, p. 39). It is of course possible that in the aecidium of *Phragmidium* with its simple structure we have a more primitive condition, and that in some other forms the association of nuclei of two different cells may have been replaced by association of daughter-nuclei of the same cell; but judgement must be suspended in the matter.

¹ Whether any protoplasm passes over with the migrating nucleus could not, of course, be determined.

MYCELIUM AND DEVELOPMENT OF UREDOSPORES.

PHRAGMIDIUM VIOLACEUM.

The uredospores appear on the leaves about June and replace the aecidia, which are only short-lived. After passing the aecidial stage the parasite appears to have gained greatly in energy, for the degree of infection is now much greater¹. The mycelium in the leaf which gives origin to the uredospores shows, naturally, paired nuclei like the aecidiospores from which it was derived.

The details of uredospore-formation were not investigated in the leaf, but from some patches on older stem-portions where the mycelium was perennial and gave rise to uredospores early in the year, even before the aecidia were developed on the leaves. These patches were found to be much more favourable objects of study than those on the leaves, the general form of which has been figured by Sapin-Trouffy. The perennial mycelium was found to form a layer of considerable thickness, in which even thin sections show a considerable number of the nuclei arranged in pairs (Fig. 78), but as the two nuclei of these cells often separate for some distance, the paired condition cannot always be observed. A number of the hyphae penetrate the cells of the host, thus acting as haustoria (Fig. 99), but they did not appear to have that special relation to the nucleus of the host-cell described for several cases by Sapin-Trouffy.

The stalked uredospores are borne on somewhat rectangular cells which may be termed *basal cells*. They grow up from the free surface of mycelium and form a regular layer (Fig. 79). These cells are usually vacuolate, and show two nuclei which are larger than the nuclei of the mycelial cells and exhibit each a well-marked nucleolus and granular chromatin. The uredospore arises from the basal cell as a binucleate outgrowth (Figs. 80, 81), which soon becomes of a somewhat oval shape. The two nuclei then undergo the process of conjugate division (Fig. 82) and the outgrowth becomes divided into two cells (Fig. 83), the upper increasing much in size and forming the uredospore, the lower remaining narrow, but elongating later and forming the stalk.

The wall of the uredospore becomes much thickened, but a very definite pit through which there is distinct protoplasmic continuity connects, for some time, its cavity with that of the slightly thickened stalk (Fig. 83 *a*). In the mature state, however, before the uredospore separates from the stalk, the pit becomes obliterated.

¹ The discovery of a process of fertilization is, of course, a confirmation of the interesting view, put forward by Arthur (1, 2), on other grounds, 'that the aecidium is a device to restore vigour to the fungus.'

The nuclei of the free uredospore have sometimes a peculiar, irregular contour as seen in Fig. 84; this appears to be only a temporary phase of development.

DEVELOPMENT OF TELEUTOSPORES.

PHRAGMIDIUM VIOLACEUM.

The teleutospores appear on the leaves towards autumn, and are at first mixed with the uredospores, but later arises in sori which consist of teleutospores only, surrounded by a layer of paraphyses. The mycelium on the leaf from which they arise shows a septate mycelium with a pair of nuclei in each cell. In Fig. 85 the paired nuclei can be distinctly seen in the haustoria in the cells marked * and in several of the other cells, but owing to the thinness of the section the nuclei appear single in a number of cases.

From this mycelium there grow up, when the teleutospore sorus is to be developed, a number of rectangular, binucleate basal cells closely packed beneath the epidermis. It is from these special cells (which Sapin-Trouffy has figured in a diagrammatic way) that the teleutospores are developed. The young teleutospore appears first as an elongated, binucleate, cylindrical outgrowth from the basal cell. It increases in diameter, and soon cuts off from the apex downwards a series of three or four superposed cells (Figs. 86, 86 *a*), which form the body of the teleutospore, the lowest cell becoming the stalk. The upper cells take on the characteristic shape, and the walls become thickened. The upper cell grows out into a short, narrow projection (Fig. 86 *b*), the cavity of which soon becomes completely or almost completely obliterated by the great increase in thickness of its wall; there is thus produced the knob-like projection characteristic of the mature spore.

The lowest cell, the stalk, increases very greatly in length, and its cavity becomes almost completely obliterated by the thickening of its walls, except in the lowest part where it is inserted on the basal cell. Here there is a cavity of considerable size in which the two disorganized nuclei are to be found (Fig. 86 *c*). Some evidence was obtained of a pit connecting for some time the end of the stalk with the basal cell (as in the case of the uredospore and its stalk); the absence of thickening at this point, and the peculiar widening of the cavity shown in Fig. 86 *c*, strongly suggest such a connexion, though its existence was not established with certainty.

During the process of thickening of the wall of the spore the nuclei in the cells show each a single well-marked nucleolus and granular chromatin, and are usually to be found close together (Figs. 86, 86 *a*). When the wall is fully thickened the process of nuclear fusion begins. In the first stage the two nuclei are found in close contact (Fig. 87 *a*); in the second stage instead of two small nuclei one larger one is found, but the two

nucleoli remain for a time without fusion (Fig. 87 *b*), as described by Sapin-Trouffy. In the next stage the nucleus has increased somewhat in size, the two nucleoli have been replaced by one large one, and the chromatin, which has hitherto not been well marked, begins to stain more deeply (Fig. 87 *c*). The nucleus still increases in size, and the chromatin becomes resolved into a very well-defined thread which is apparently a single spireme (Fig. 88 *a*). This, however, is not the precursor of division, for the nucleus still increases in size and the chromatin returns to the condition of a fine network with the nucleolus as a somewhat elongated body flattened against the nuclear membrane (Fig. 88 *b*). The nucleus then goes into the resting state, in which it passes the winter, appearing as an almost homogeneous body except for the well-marked nucleolus (Fig. 2). The process of increase in size and of formation of a spireme thread seems to correspond with the changes included under the term *synapsis* in the higher plants and animals, but it is not followed by nuclear division but by a period of rest in which the spireme again disappears. A somewhat similar case has been described by Williams (60) for the tetraspore-mother-cell of *Dictyota*, where after *synapsis* a similar disappearance of the spireme thread and a period of rest was found to occur.

GYMNOSPORANGIUM CLAVARIAEFORME.

The perennial mycelium which inhabits the stems of *Juniperus* and bears in spring the yellow masses of teleutospores shows very clearly the paired nuclei in the cells of hyphae, which are thick-walled as described by Sapin-Trouffy and others. As in the case of the teleutospores and uredospores of *Phrag. violaceum*, the teleutospores of this form are not borne directly on the mycelium but arise from comparatively large rectangular cells, which form a close-set layer on the surface of the mycelium, at the points where the teleutospores are developed. These each contain two nuclei which are somewhat larger than those of the ordinary cells of the mycelium, and show each a well-marked nucleolus (Fig. 89). They are similar to the teleutospore-bearing cells described by Sapin-Trouffy for *G. Sabinae*, but their side and lower walls are of considerable thickness. Each of these cells gives origin to a number (not more than three or four) of narrow outgrowths which develop into the stalked, two-celled teleutospores (Fig. 89). The outgrowths have paired nuclei (Fig. 90) and soon undergo two divisions, the first cutting off the stalk-cell, while the second divides the upper cell into the two cells of the teleutospore. The teleutospore then increases in size, the wall becomes thickened, and the cytoplasm vacuolar and filled with yellow, oily reserve-material.

The two nuclei in the teleutospore fuse, earlier than in *Phragmidium*, before the spore has obtained its full size or thickness of wall. The wall

at the point of contact of the two nuclei disappears and the two nuclei fuse, the two nucleoli being distinguishable for some little time after fusion (Fig. 91 *a, b*, and *c*). The fusion-nucleus then increases in size and goes through changes exactly similar to those of *Phragmidium*. A single large nucleolus appears, and the chromatin becomes resolved into a thick, continuous, spireme thread (Fig. 92). Here, also, this condition is merely temporary, for the thread after a time becomes replaced by a fine network, with usually only small inconspicuous nucleoli (Figs. 14 and 15), as described under the mature teleutospore. As in the case of *Phrag. violaceum*, these nuclear changes must be looked upon as a process of *synapsis*.

METHOD OF 'CONJUGATE' DIVISION.

The method of division of the paired nuclei is of the same simple type as that described for the single nuclei of the spermatial hyphae, but the two nuclei are always to be found in the same stage of division and in very close association during the process. This division can best be observed in the development of the aecidiospores, uredospores, and teleutospores.

The first step in the process is the disappearance of the nuclear membrane and the condensation of the chromatin of each nucleus into a homogeneous, irregular mass, with the natural result that the single nucleolus (which is always present at least in the nuclei of the cells connected with spore-formation) comes to lie free in the cytoplasm (Figs. 93 *a*, 95 *a*). Sometimes the chromatin can be observed in a state where it is not yet completely homogeneous and still retains in part the form of the original nucleus (Fig. 98 *a*). In the next stage the chromatin mass becomes more regular (Figs. 93 *b*, 95 *b*), and in favourable cases can be seen to be connected with a thread-like structure (Figs. 72, 93 *b*), which has the staining reactions of kinoplasm, and is no doubt to be considered as of the nature of a simple spindle. The chromatin mass then becomes elongated to form a thick rod which is apparently stretched out on the spindle, for the latter is completely obscured (Figs. 93 *c*, 95 *c*). Each chromatin rod then becomes drawn out into two more or less pear-shaped masses (Figs. 93 *d*, 95 *d*), which at first remain connected by a fine thread which has the staining reactions of kinoplasm (it takes the gentian in the triple stain), and is no doubt the drawn-out spindle (cf. divisions in promycelium). As the two masses move still further apart the connecting thread becomes very faint and ceases to be continuous (Fig. 93 *d*). At this stage, or somewhat earlier, the rejected nucleoli, which have hitherto lain almost unaltered in the cytoplasm, begin to decrease in size and finally disappear, though they may remain visible up to the stage in which the new nucleoli have begun to appear in the daughter-nuclei (Fig. 93 *e*).

In some cases, as in the case of the divisions of the single nuclei, it can clearly be observed that the chromatin does not form a single rod but a double rod (Fig. 96), which becomes drawn out into *two* pairs of chromatin masses, so that at this stage of the anaphase the nuclei show two opposite groups, consisting of four chromatin masses (Figs. 97 and 98). How far the presence of these two masses in the nucleus is of common occurrence it is difficult to say, for if the plane of separation of the two lies at right angles to the direction of view they would easily be overlooked. There would seem to be no doubt, however, that they are only occasionally present in the divisions in *Phragmidium*; in the teleutospore in *Gymnosporangium*, however, the double chromatin mass seems to occur more frequently.

It is the appearance of these two chromatin masses which led Sapin-Trouffy (48) and Maire (29, 30) to the belief that the nuclei in the Uredineae possess in all cases two chromosomes, while Poirault and Račiborski (43), observing the single mass, considered it to represent a single chromosome. It is obvious, however, that neither the origin nor behaviour of these structures is that of chromosomes, and the observation of numerous chromosomes, described earlier for the promycelial divisions, is sufficient to negative such a view. The real nature of these chromatin masses is, however, apparent on comparison of the divisions in the promycelium with the simpler divisions, either single or paired (conjugate), found in other stages of development. In both the promycelial divisions the chromatin (vide supra) shows at times a distinct segregation into two masses (Figs. 30, 32, 41 a). These masses are certainly not themselves chromosomes, and in the first division are clearly produced by the aggregation of numerous chromosomes; they probably represent, as suggested earlier, the chromatin derived respectively from the two nuclei which fuse in the teleutospore. It is not surprising then that nuclei which are the direct descendants of those in the promycelium should also show a similar tendency to a differentiation of their chromatin into two masses, though they have apparently ceased to form chromosomes. A comparison of Fig. 30 with Figs. 94 and 96 shows clearly the strong resemblance between the form taken by the chromatin in the two types of division.

NATURE OF SPERMATIA.

As the study of *Phrag. violaceum* clearly shows, the spermatia take no part in aecidium-development, and fertilization is regularly brought about in another way. This result is only in agreement with all earlier work, which has always failed to establish any direct relation between the spermatia and the aecidia. A fertilization of the *fertile cells* would obviously be difficult in *Phragmidium*, but it would be almost impossible in the typical aecidium, where the fertile cells are developed deeper down in the tissues.

As the spermatia have no power to act as male cells and appear quite incapable of causing infection, there would seem to be no escape from the view that they are now *functionless*¹. It becomes evident then that a study of their structure is the only means by which one can hope to obtain evidence as to their primitive nature, for originally they must have acted either as male cells or as conidia. Strangely enough, their histological characters have never been considered from this point of view, though they appear to give very definite evidence for a decision of the disputed question.

As the observations detailed earlier show, the spermatia, in both the forms investigated, are small, uninucleate cells, with a thin wall, apparently no reserve-material, a very dense nucleus without a distinct nucleolus, and a very small amount of cytoplasm (Figs. 47, 59)². That these characters are general throughout the whole group is shown by a glance at the figures of Sapin-Trouffy (48), diagrammatic as they are; though this observer, assuming that the spermatia were conidia, passed over these characters without particular comment. It is obvious, however, that these characters are not those of conidia nor of any regular asexual reproductive cells; on the other hand they are to a very striking degree the characters of *male* cells, in which, as is well known, there is usually little or no reserve-material, the nucleus is often very dense, and the cytoplasm is nearly always much reduced in amount.

The reduction in cytoplasm is very well marked in the small spermatia of *Phragmidium* and *Gymnosporangium*, but it is just as clearly shown in the comparatively large spherical spermatia of *Coleosporium Senecionis*, Fr., where, according to the figure of Sapin-Trouffy, the diameter of the nucleus is more than two-thirds of that of the whole cell. Such a structure is certainly without parallel in any known asexual reproductive cell, and sufficiently explains the inability of the spermatia to develop in water, for the volume of cytoplasm is insufficient to form a germ-tube of any length.

When one considers the peculiar structure of the spermatia of the Uredineae, their total incapacity, as far as is at present known, to bring about infection, their feeble power of development even in nutritive solutions, their usual close association with the aecidium, there seems no escape from the view that the *spermatia are male cells which have now become functionless*. The peculiar process of fertilization in the fertile cells of the aecidium of *Phrag. violaceum*, which one can hardly but consider as a reduced process, seems only explicable on the view that the spermatia formerly acted as fertilizing agents in connexion with the aecidia.

¹ It is satisfactory to note that Klebahn (27) in his recently published work, though unable to throw any light upon their nature, also arrives at the conclusion that they act neither as conidia nor male cells; he thinks they may in some way be useful to the plant as an excretory product.

² I have also observed the same structure in the spermatia of *Puccinia Poarum*, Niels, *P. Phalaridis*, Plowr., and *Uromyces Poae*, Rabh.

It must be pointed out in relation to the observations of Cornu (12), Brefeld (10), Plowright (42), Sapin-Trouffy (48), and others on the germination of the spermatia in nutritive solutions, that a complete absence of power of vegetative development is not a necessary character of male cells, as is shown by the well-known cases of the potential gametes of some algae which can develop either sexually or asexually. It must also be remembered that nutritive solutions are a highly artificial condition for the reproductive cells of such obligate parasites as the Uredineae; it is difficult to understand how the spermatia could act in nature as infecting organs (conidia) unless they are able to germinate in water like the other spore-forms of the group. Their appearance at about the same time as the *aecidiospores* is another argument against their conidial nature (as well as an argument in favour of their sexual nature), for, in the presence of such very effective infecting organs as the aecidiospores are known to be, there would seem to be absolutely no need for the production of conidia so structurally ill-equipped for directly carrying on the life-cycle as are the spermatia. The fact that most of the spermatia in the forms investigated are found to be disorganized soon after they are shed, and that rarely in *G. clavariaeforme* some of them degenerate while still in the spermogonium, is evidence against the conidial view and in favour of the view that they are abortive male cells.

There is of course a view which might be suggested, that the spermatia are degenerate conidia which have ceased to have any function. Such a view, however, may certainly be dismissed, for it is difficult to imagine the conditions in which the conidia would cease to be of value, and still more difficult to imagine a process of degeneration which brought about a reduction of the cytoplasmic portion of the cell, but left the nucleus untouched.

That the spermatia, though perfectly functionless, should still be produced by the majority of forms and in such great abundance is certainly a very striking phenomenon¹. A somewhat similar process, apparently equally wasteful, is to be observed in certain animals, as in the gasteropod, *Paludina*, where two sorts of spermatozoa, normal and giant ones, are constantly produced, although the smaller ones only are functional.

The similarity of the spermogonia of the Uredineae to those of *Collema*, in which the earlier observations by Stahl on the fertilizing action of the spermatia have of late years been confirmed by Baur (5), is additional confirmation of the view that the spermatia in the Rusts are male cells.

It is true that Möller has shown that in certain cases the spermatia

Even when the aecidium is dropped out of the life-history the spermogonium sometimes still remains as the first structure to be developed on the mycelium which arises from the sporidium, being later followed by uredospores or teleutospores; e. g. in the so-called *brachy-* forms.

of lichens can develop as conidia, but, as pointed out by E. Fischer (Bot. Zeit., 1888, p. 158) in reviewing Möller's work, and also later by Harper (20), this in no way proves that the spermatia are not potential male cells. That they do not usually develop except in nutritive solutions, that it is often months before they germinate, and that their growth is excessively slow, strongly suggest that they are not simple conidia; but these facts are quite in keeping with the view that they are male reproductive elements. The suggestion lately put forward by Metzger (33)—that the spermatia of lichens are male cells which have retained a certain power of vegetative development, and, now that in many cases the ascus fruit develops without their aid, they sometimes act as conidia, and may have become modified in that direction—seems to be the most satisfactory one¹.

There seem to be no data for a comparative cytological study of the spermatia of lichens, though it would be of great interest. The figure of the spermatium of *Buellia punctiformis* given by Istvanffi (24) shows a cell with a much smaller nucleus in proportion to the size of the cell than in the Rusts; but it is possible that only the nucleolus was observed.

FERTILIZATION IN THE AECIDIUM AND THE NUCLEAR FUSION IN THE TELEUTOSPORE.

It is very clear that the process which takes place in the young cells of the aecidium of *P. violaceum* is one which has most of the characters of an ordinary sexual process. A cell is to be observed, which after cutting off a sterile cell above, increases in size and becomes a specialized reproductive cell, exhibiting abundant protoplasm and a large nucleus with a well-marked nucleolus. A pause then occurs in its development, but it is later stimulated to further growth and rapid division by the entrance of a nucleus from without.

Such a series of phenomena leave no escape from the view that the fertile cell is a *female* reproductive cell which undergoes a process of *fertilization*. There are, however, two points in which the process differs from that of ordinary fertilization. One is, that the two nuclei which become associated in the fertile cell do not fuse, but retain their morphological individuality, though the closeness of their relationship is shown by their always dividing together in close juxtaposition; a fusion of nuclei does ultimately take place in some of their descendants, but it is confined to those pairs of nuclei which are to be found in the teleutospores. The second point of difference is, that the entering nucleus is derived from an un-

¹ Hedlund (22) has described two cases in which the spermatia of lichens germinated and developed a thallus in a state of nature.

differentiated vegetative cell and not from a specialized male cell or organ; this will be discussed in the next section.

A study of the cytological features of fertilization in various animals and plants shows clearly that *actual fusion of nuclei is not a necessary part of fertilization as ordinarily understood*. Although in Angiosperms the sexual nuclei fuse together before the egg develops further, yet in the Abietineae (Blackman, 6; Ferguson, 16), and in most animal eggs, development of the egg begins without the fusion of the nuclei, for the two begin to divide independently and only meet together on the first division-spindle. Again, in the egg of *Cyclops*, we have cases in which the two nuclei can be distinguished for several cell-generations¹. Not only was Rückert (46) able to observe that in the first segmentation of the egg of *Cyclops* the two nuclei divide side by side in an association very little closer than that to be observed in the conjugate nuclei in the Uredineae; but Häcker (17, 18) even observed *double* nuclei in the cells of the embryo up to the sixteen-celled stage (at what stage actual fusion took place was not determined).

Again, in many cases of fertilization the fusion between the two nuclei is more apparent than real, for the chromatin and chromosomes derived from the male and female nuclei respectively have in a number of instances been traced as separate structures through several generations (eggs of *Ascaris*, *Cyclops*, &c., and the chromosomes in *Pinus*). Häcker (17) also believes that in the egg of *Cyclops* he has been able to trace the maternal and paternal chromatin as separate structures from the time of fertilization up to the formation of the next germ-cells. In fact all the cytological work of recent years tends to show that the chromosomes have a distinct 'individuality,' and that the nuclei of the cells of the higher animals and the nuclei of the sporophytic cells of the higher plants are really *dual* in nature, there being no real mixing of the chromatin from the two sources until the time of chromosome-reduction².

A study of the varieties of fertilization in the higher animals and plants shows clearly that three morphological stages are to be observed in connexion with the complete sexual cycle—*nuclear association within the*

¹ Conklin (11) also in the egg of *Crepidula* was able to observe the double character of the nuclei at the telophase in every cleavage up to the twenty-four-cell stage, and in several cleavages up to the sixty-cell stage.

² See for example the work of Van Beneden, Boveri, Herla and Zoja, and of later years that of Sutton (52), and the observations on chromosome reduction of Montgomery (37, 38), Sutton (51), and Farmer and Moore (15). Sutton describes a very interesting case in which the chromosomes (twenty-three in number) in the spermatogonium of an insect (*Brachystola*) are not only of eleven different sizes, those of the same size being arranged in pairs, but the majority of them in the resting state are each contained in a separate diverticulum of the sacculated nucleus, the 'accessory chromosome' lying in a separate closed vesicle and thus forming, virtually, a separate nucleus.

The results obtained in certain hybrids in which the sexual cells are apparently 'pure' in relation to certain maternal characters also suggest that the nuclear material from the two sources remains distinct from the time of fertilization up to the formation of the germ-cells.

same mass of cytoplasm and during division, nuclear reduction (fusion), and chromosome-reduction. These processes may either take place together or be separated by a considerable number of divisions. In the case of the higher plants the first two processes usually take place together, i. e. the sexual nuclei fuse in the resting state directly they meet¹. In many animal eggs, and a few plants, the second process is somewhat delayed, and there is no nuclear fusion until the end of the first division. In the case of the egg of *Cyclops* we have a further stage, for nuclear reduction is put off for many cell-divisions, each of the cells showing two nuclei which are, however, closely associated together during division. In the Uredineae there is a still further stage of separation, for in this case nuclear reduction (or fusion) is put off until the stage corresponding with chromosome-reduction (vide infra), so, like that process, it is confined to the reproductive cells (teleutospores = spore-mother-cells) which are to carry on the life-history. The two nuclei, also, instead of dividing together on the same spindle divide on separate (rudimentary) spindles side by side.

Fertilization is, of course, a somewhat ill-defined term, and from a strict morphological point of view it is perhaps arbitrary to confine it to any one of the three processes just described, for sooner or later all of them must occur in the complete sexual cycle. A cell is, however, usually considered to be 'fertilized' directly the stimulus has been given to development and the number of chromosomes doubled by the entry of the male cell or nucleus, and for such a use of the term fertilization it is obvious that the essential point is the first stage—nuclear association in the same mass of cytoplasm and during division.

The time at which nuclear reduction (fusion) takes place is seen from the stages given above to be quite unimportant, but, like chromosome-reduction, it must occur sooner or later in the sexual cycle. The view which would consider fertilization as incomplete until nuclear fusion had taken place could hardly be accepted, as chromosome-reduction might equally be considered as the end of the process. It would also tend to divorce the morphological and physiological aspects of fertilization, for in *Cyclops* and *Phragmidium*, for example, we should have to consider the female cell as developing under the stimulus of fertilization, but with the process still incomplete. Besides, all recent work at present tends to show that nuclear fusion is mainly a nominal process (hence the term nuclear reduction is preferable), a mere change from association within the same cytoplasm to association within the same nuclear membrane.

The view of Dangeard and Sapin-Trouffy, which would consider the fusion in the teleutospore as itself a sexual process, was based on an

¹ It is generally believed that in many of the Thallophyta where there is no alternation of generations the *three* processes all take place together, chromosome-reduction occurring before the fusion-nucleus divides.

ignorance of the origin of the two nuclei and the exaltation of nuclear fusion as a test of fertilization. The fusion is the result of a sexual process (at least in *Phrag. violaceum*), but is clearly not in itself a fertilization. Račiborski (44), when he put forward the hypothesis that the conjugate divisions in the Uredineae represented a vegetative phase intercalated in fertilization between the stages of cytoplasmic fusion and nuclear fusion, was much nearer the truth, though, as has been pointed out above, it is not at all necessary to consider nuclear fusion as a stage in fertilization as usually understood. Račiborski, equally with Dangeard and Sapin-Trouffy, was quite ignorant as to the method by which the two nuclei became 'conjugate.'

Maire (29) had earlier recognized a similarity between the conjugate nuclei of the developing egg of *Cyclops* and the paired nuclei of the Uredineae, and applied the term 'synkaryon' to the two nuclei. He recognized that the fusion in the teleutospore was not in itself a process of fertilization, but considered it a mere process of 'mixie,' in which he includes both nuclear and chromosome reduction in the sense used above. He believed the 'synkaryon' to be brought about by the association of two daughter-nuclei of a cell, and at first considered it actually comparable to a sexual process, but in a later paper (30) he comes to the conclusion that between the fertilization in the higher plants and the formation of the 'synkaryon' in Uredineae (and Basidiomycetes generally) there are only 'des relations de cousinage.' He elaborated his views in some considerable detail, but the discovery of the origin of the two nuclei in the aecidium of *Phragmidium*, and the evidence here brought forward that the spermogonia and aecidia are in all cases to be considered as sexual organs, throws a completely new light upon the matter; his views need not therefore be discussed further.

ORIGIN OF THE PROCESS OF FERTILIZATION.

The process of fertilization as observed in the fertile cells of *Phrag. violaceum* might be looked upon as merely one of great simplicity in which the male cell remained undifferentiated. The two nuclei which take part in the process are not sister-nuclei though they may sometimes be closely related, and the process as a whole (except for the absence of obvious cytoplasmic fusion) is not much simpler than that of *Basidiobolus*, in which two neighbouring cells, after forming each a sterile cell, fuse together to form a zygosporangium.

Taking into account, however, on the one hand, the completely undifferentiated nature of the cell from which the acting male nucleus comes, and the peculiar method by which the nucleus passes from one cell to another, and on the other, the presence of the functionless spermatia with their special cytological characters, the only view that seems satisfactory to explain the facts is *that the primitive normal process of fertilization by*

means of the spermatia has been replaced by a fertilization of the female cell by the nucleus of an ordinary vegetative cell.

Such a view receives strong support from the observation of Farmer, Moore, and Digby (14) on apogamy in Ferns, published while this work was in progress. There seems no doubt that the process in the aecidium is to be looked upon as intermediate between that of normal fertilization, where both the cells are specially differentiated, and that observed in apogamous Ferns by these observers, where both acting male and female cells are represented by ordinary vegetative cells.

If the view be accepted that the process observed is a reduced form of ordinary fertilization, it seems very probable that the sterile cell also is reduced. Its position above the fertile cell would suggest that it formerly acted as a receptive cell pushing up between the epidermal cells as a trichogyne to which the sticky spermatia could be brought, for example, by insects. Some support is lent to such a view by the fact that occasionally cases are to be found in which the sterile cells do push up between the epidermal cells and swell out above, being merely covered by the cuticle (Fig. 77 a). If development were pushed one stage further and the cuticle pierced, a very effective receptive organ would be the result.

MORPHOLOGY OF THE AECIDIUM.

Although this subject cannot be treated fully in the absence of a comparative study of aecidium development in various members of the group, yet, as has been stated earlier, the aecidium of *Phragmidium* (and apparently that of *Caeoma*) is very different from the typical aecidium found in the other genera. It is merely a group of special reproductive organs, indefinite in extent, and merely bounded by paraphyses which are sometimes absent; and thus no more a definite structure than the sorus of uredospores or teleutospores.

In the light of our present knowledge the aecidium of *Phrag. violaceum* is thus to be considered as a group or sorus of female reproductive organs, each consisting of a female (fertile) cell below and a sterile cell above.

We probably have in this genus the most primitive form of the aecidium to be found in the group, but of the form of the aecidium in the still more primitive state when fertilization by the spermatia yet occurred, we can say nothing¹. With the change to the reduced form of fertilization, with its obvious advantage of certainty, the number of female organs in the group could be indefinitely extended without the necessity of breaking through to

¹ It is of course possible that the spermatia may sometimes really act as fertilizing agents in *Phragmidium*, and perhaps in *Caeoma*, for they are occasionally found scattered over the surface of the leaf below which the aecidium is developing; and if a sterile cell should push through to the surface and come in contact with a spermatium fertilization might result: such a process, however, was never observed, and it can only be of very rare occurrence.

the surface. The absence of this necessity might also lead later to a development deeper down in the tissues in the host, as is found in the typical aecidium, where conditions of nutrition would perhaps be more satisfactory. With this deeper point of origin may also perhaps be associated the development of the pseudoperidium, which would protect the young structure while pushing its way to the surface.

There can be little doubt that the aecidium, when present, is always the seat of the process of transition from the condition of single to that of paired nuclei. Sapin-Trouffy (48) found this to be the case in all the forms (about fourteen) which he investigated. His observations have been confirmed by Maire (29; 30) for *Endophyllum Sempervivi* and for *Puccinia Bunii*, DC., and by the writer in the two forms here described, and also in *Puccinia Poarum*, Niels, *P. caricis*, Rebent, and in *Uromyces Poae*, Rabh. The conclusions as to the nature of the aecidium in *Phragmidium* must then apply throughout the group, and the aecidium in all cases must be considered as a group of female reproductive organs¹. Whether in all cases the transition (fertilization) is brought about by a nuclear migration, or whether in some cases the two nuclei are formed in the cell by division (as Maire believes), must be left to future work to decide. Such a condition would, however, be merely a further stage of reduction in fertilization, comparable to the well-known case of fertilization by means of a sister-nucleus (the second polar body) observed in the 'parthenogenetic' eggs of *Artemia*.

The hypothesis put forward by Poirault and Račiborski, that the sporidium may become binucleate and so start a mycelium with paired (conjugate) nuclei, is quite untenable. The work of Sapin-Trouffy, of Maire, and the observations here detailed have shown that in all cases investigated the mycelium which arises from the sporidium has single nuclei. It is true that in certain cases, such as in *Coleosporium Euphrasiae*, investigated by

¹ In *Coleosporium* what are usually known as the uredospores are produced in chains and have intercalary cells between them, and are thus liable to be taken for the true aecidiospores though no pseudoperidium is present. In forms such as *C. Senecionis*, Fr., in which the full life-history is known (about fifteen species of this genus are now known to be heteroecious), there can be no confusion between the two, as the true aecidium is found early in the year on the one host, is associated with spermogonia, and has a definite pseudoperidium; while the aecidiospore-like uredospores are produced later, on the other host, and are intercalated, like ordinary uredospores, in the life-cycle between the true aecidiospores and the teleutospores. Sapin-Trouffy has shown, as was to be expected, that in *C. Senecionis* the transition from the single to the paired nuclei takes place in connexion with the true aecidium, while the aecidiospore-like uredospores are borne on a mycelium with paired nuclei derived by infection from the aecidiospores. Owing to their position in the life-cycle, the histological nature of the mycelium which bears them, and the absence of a pseudoperidium, it is clear that the spores in question partake much more of the nature of uredospores than of aecidiospores, so the former name should be retained for them in spite of their development in chains. Bearing in mind the heteroecious nature of nearly all the forms of *Coleosporium* hitherto investigated the view put forward by Holden and Harper (23) can hardly be accepted, that the uredospores are really aecidiospores in the form to which they give the name *C. Sonchi-arvensis*, Lév., and of which they observed only the stage with uredospores and teleutospores.

Poirault and Raciborski, the sporidium does become binucleate, but this appears to be a mere precocious division of the nucleus without wall-formation, for, as mentioned earlier (see under section dealing with sporidia-formation), the sporidia of *Phrag. violaceum* and a few other forms have been found to give origin, in spite of their binucleate nature, to the normal mycelium with single nuclei. Poirault and Raciborski, however, observing that the mycelium which gave origin to the uredospores and teleutospores had paired nuclei and that the sporidium also was binucleate, concluded that the former arose from the latter. They completely, however, overlooked the fact that *C. Euphrasiae* had been shown by Klebahn (26) to be a heteroecious form and that the sporidium really gave rise to the aecidial stage, the so-called *Peridermium Pini*, which in the case of *C. Senecionis* Sapin-Trouffy had shown to have the typical structure, a mycelium with single nuclei. Holden and Harper (23) in a recent paper, observing that the sporidium becomes binucleate in a form to which they give the name of *Coleosporium Sonchi-arvensis*, Lév., have again put forward the view that the sporidium starts the stage with paired nuclei. Such a view, for the reasons just given, can hardly be accepted without direct evidence. The authors assume that the binucleated sporidium gives rise directly to the mycelium with paired nuclei which they investigated and found bearing the uredospores and teleutospores; but it has been shown that the species in question, like that of *C. Euphrasiae*, is heteroecious (see Klebahn, 27), with its aecidial stage on *Pinus*, like nearly all the other species of the genus yet investigated. The aecidial stage in this form has therefore no doubt a mycelium with single nuclei like *C. Senecionis* and all the aecidial stages of the Uredineae hitherto known. The mere fact of the sporidium being binucleate cannot be considered as being of any special significance. It is true that the name *C. Sonchi-arvensis* has been applied loosely to a number of what are now known to be different forms, the original form being really confined to *Sonchus* in its second stage (see also Klebahn, 27). The *Coleosporium* on *Aster* and *Callistephus* will probably thus prove to be a new form, but there can be no reason to doubt its heteroecism, since nearly all the forms of the genus hitherto investigated appear to have two hosts.

There is no evidence that the sporidium of any form ever gives rise to anything but a mycelium with single nuclei. As stated earlier, all the work of Sapin-Trouffy, of Maire, and that here detailed goes to show that the condition with paired nuclei always starts in the aecidium when that structure is present. Even in the absence of the aecidium the paired nuclei only arise *later* in the mycelium in connexion with the development of the uredospores or teleutospores¹.

¹ Arthur (61), in reviewing the work on this subject, concludes that the sporidium regularly starts the condition of paired nuclei in the group, and is naturally at a loss to explain the fact that the spermatia are uninucleate. His views seem based on a misunderstanding of the work of Sapin-

NUCLEAR DIVISION.

From the observations of Poirault and Raciborski, Sapin-Trouffy, and Maire—although they did not observe all the details of the process and their interpretation is clearly at fault—there can be no doubt that the type of nuclear division here described, whether for single or conjugate nuclei, is general for all the Uredineae. It is clear then that in all of the divisions in the group, except those in the promycelium, we have to deal with a process of such a simple nature that chromosome-formation is in complete abeyance; so that the division actually partakes of the nature of direct division (amitosis). A comparison, in such a form as *G. clavariaeforme*, of the first and second divisions in the promycelium with the other simple divisions (whether single or conjugate), leaves no escape, however, from the view that the simple method of division is really a *reduced* form of *indirect* division. The first promycelial division is a fairly typical form of mitosis with a formation of chromosomes, though the absence of any regular equatorial plate, the fact that no splitting can be observed, and the early fusion of the chromosomes suggest that perhaps even here the process may be reduced from a halving of definite chromatin elements to the more or less direct separation of chromatin material as a whole. In the second division, however, though the spindle structure is perfectly typical, the chromatin instead of forming distinct chromosomes merely forms a network which becomes spread out on the spindle and later draws apart into two portions¹. If this second method of division be still further reduced, so that the chromatin instead of forming a network forms a solid mass and the spindle is represented only by a fine, thread-like structure, we have the ordinary method of division characteristic of the nuclei, whether single² or paired, of the cells other than those of the promycelium.

It seems impossible to doubt that these three methods of division represent progressive stages in a process of reduction, for it is hardly

Trouffy, who showed clearly (and his work has been confirmed by Maire and in this paper) that the spermogonium was always borne on a mycelium with *single* nuclei. Arthur also states that it has been shown that both acidiospores and uredospores arise from a binucleated mycelium in the usual vegetative manner. This is directly contrary to the facts observed by Sapin-Trouffy eight years ago, who in all cases found that the acidiospores were borne on a mycelium with single nuclei, and that the nuclei only became paired at the time of acidium-formation.

¹ Whether the two promycelial divisions are regularly different in other forms as in *G. clavariaeforme* remains for future work to show. Holden and Harper (23), in their recent paper, are of the opinion that in the species of *Coleosporium* which they examined the two divisions are of like nature, though they were not fortunate enough to have such a complete series of stages as were obtained for the form here investigated.

² In the divisions of the single nuclei, as observed in the spermatial hyphae, the spindle structure is not so clear. A precocious division of the nucleus of one of the four 'promycelial' cells has been observed, however (while this paper was in the press), in *G. clavariaeforme*, which shows a distinct spindle, though without centrosomes or polar radiations (Fig. 100). This shows that the achromatic part of the nucleus may not always be as reduced as in most of the divisions observed.

conceivable that in such a highly developed group as the Uredineae we should witness the evolution of mitotic division. The second division with its very complex achromatic mechanism but simple method of chromatin segregation points clearly to the process being a reduced one.

A comparative study of nuclear division in the Uredineae with that to be observed in the Basidiomycetes would be of great interest, and, considering the obvious relationship of the two groups, might throw some light on the question as to whether the reduction in nuclear mechanism in the former group is to be connected with the marked parasitic habit. The two chromosomes described by Maire (30) as universal for the Basidiomycetes are doubtless merely chromatin masses, comparable to those described above. Wager (55, 56), Ruhland (47), and Harper (21) have all shown that in the forms they investigated the chromosomes in the basidium were much more numerous. Maire seems to have observed such structures in certain cases, but he applies to them the term 'protochromosomes,' and states that they fuse later to form the two structures which he believes to be the real chromosomes. This is exactly what happens in some cases in the promycelium in *G. clavariaeforme* (Fig. 30), but there can be no doubt, as the figures show, that the first-formed structures are the true chromosomes.

With reference to the question of the function of the nucleus and the relation of direct to indirect division, the existence of a group of Fungi, which in the whole life-cycle show but one, or perhaps two, divisions in which there is a distinct formation of chromosomes, is certainly a very interesting fact. The view that direct nuclear division is confined to cells which are in a senile state has of late years been largely modified. It has been shown that a number of Protozoa and certain animal cells divide normally in an amitotic manner, and Nathansohn (39) has shown for *Spirogyra*, and Shibata (50) for *Podocarpus*, that cells which have divided amitotically can later divide in the ordinary mitotic manner. Similarly Häcker (19), Wasielewski (58), and Němec (40) have shown that nuclei which have divided in an abnormal¹ way under the influence of narcotics can again divide in the normal indirect manner when placed under natural conditions.

These observations are generally considered to show that the nucleus has remained entirely unaffected by the intercalation of one or more direct divisions in the normal series of mitoses, but they really prove no more than that the effect (if any) has not been sufficiently profound to destroy their power of normal division or to prevent them carrying on their normal development within the limits of the experiment. Němec has pointed out

¹ There seems considerable doubt as to how far these divisions are abnormal. Wasielewski considers them to be of the nature of amitoses, while Němec brings forward very considerable evidence to show that they are merely modified indirect divisions in which, however, there is still an exact halving of the chromatin.

that in the case of the lower organisms, the effect of a division, presumably unequal, of the nature of amitosis might be long delayed; and in the case of the higher plants might not be visible immediately. It seems quite possible, however, that even in the case of the higher plants a change might be produced which would not be observable at all in the ordinary restricted life-history of cells other than the germ-cells. We have no knowledge as to how unequal an indirect division may be in relation to the chromatin; no doubt the degree of inequality must be very variable (it may probably be sometimes as equal in effect as indirect division), but it is quite conceivable that the change produced by a direct division in the 'hereditary properties' of, say, a root-cell may be confined to those concerned with flower or leaf development, and so never be visible in the normal course of development of a root-cell.

The beautiful experimental researches of Boveri (9) on double fertilized eggs of the Sea-urchin (in which he practically proves the existence of physiological individuality in the chromosomes) lend strong support to such a view, for certain blastomeres of the three- or four-celled stage, though capable of normal division and apparently similar to the other blastomeres, were found later to be deficient in the power of producing certain organs (such as the skeleton, pigmentation, &c.), owing to the unequal sorting of the chromosomes in the first division.

It is clear however that in the case of the intercalation of an amitotic division in the series of divisions leading directly up to the germ-cells, such as that described by Meeves (32) and Macgregor (31) for the spermatozoa of Amphibians, the effect of any inequality of division would produce an effect, sooner or later, in the offspring. If such a division be confirmed it seems hardly possible to consider it as other than a true equational one of equal value with mitosis; at least if the ordinary view be accepted as to the meaning of chromosomes and their splitting. There seems to be some doubt, however, as to whether this form of division is regularly present. Sutton (52) has also suggested that, as the amitotic division in these cases does not apparently lead to cell-division, it may be that the two nuclei meet again on the spindle of the next mitosis, and so the direct division is without effect.

The view may perhaps be hazarded that the sufficiency for the cell needs of the Uredineae of such a simple method of division may be connected with the simple organization of such forms as compared with the higher plants; the 'idioplasm' must be far less complex.

ALTERNATION OF GENERATIONS.

If the view be accepted that the spermogonia and aecidia represent male and female organs respectively (and the observations in *Phragmidium* appear to leave no escape from such a view) it is clear that the Uredineae

with the aecidium in their life-cycle exhibit an alternation of generations as sharply marked as that of the higher plants. For not only are the two generations to be distinguished as sexual and asexual—bearing in the one case the sexual organs, spermogonia and aecidia, and in the other case only asexual spores, aecidiospores, uredospores, and teleutospores—but they are also to be cytologically differentiated, the sexual generation being characterized by the presence of single nuclei, the asexual by the presence of paired (conjugate) nuclei; the special nuclear condition of the sporophyte being the result, as in the case of the $2n$ chromosomes of the higher plants, of a process of fertilization (at least in *Phrag. violaceum*).

Owing to the extreme reduction in the process of nuclear division in the Uredineae, so that the process of chromosome-formation is in abeyance in nearly all the divisions, one cannot distinguish the two generations by their number of chromosomes; yet one can distinguish in the nuclei of the oophyte *two* chromatin masses, while the two paired nuclei, which together correspond with the single nucleus of the ordinary sporophyte, show *four* chromatin masses. There is thus a near approach to the distinction of chromosome number which is to be found in the higher plants, and also in the interesting alternation of generations which Williams (59, 60) has lately observed in the Dictyotaceae.

The teleutospore clearly corresponds to the spore-mother-cell, for it is there that the return to the nuclear condition characteristic of the oophyte is brought about. In the teleutospore, although no actual reduction of chromosomes can be observed, there is a reduction from the four chromatin masses of the two nuclei to the two chromatin masses of the single nucleus, each mass, as shown above, appearing to correspond to a group of chromosomes. This reduction in number of chromatin masses is also associated with peculiar changes in the nucleus concerned which correspond to the process of *synapsis*.

As however the two nuclei remain individualized from the time of fertilization up to the time of reduction in number of chromatin masses we have associated with this process a *nuclear reduction*, i.e. a reduction in the number of the nuclei from two to one. Since this process of nuclear reduction takes place in most organisms at the time of fertilization, or soon after, it has been confused with that process itself, and so has led to the belief that the fusion of nuclei in the teleutospore was itself a sexual process. This view, which was hardly credible even on the facts hitherto known, has been rendered quite untenable by the discovery of an association of nuclei in the aecidium, which must itself be considered as the fertilization process.

After the process of synapsis and chromatin-reduction, the cell of the teleutospore undergoes a process of tetrad division to form the four cells of the 'promycelium' (= spores). The teleutospore (or its cell) is thus exactly

comparable to the spore-mother-cell of the higher plants and to the tetra-spore-mother-cell in the Dictyotaceae. The gametophyte generation starts again in the teleutospore and is continued up to the 'fertile cell' (the cell bearing the mother-cells of the aecidiospores) in the aecidium; while the sporophyte continues throughout the rest of the life-history, through the aecidiospores, the uredospores (if present), and up to the teleutospores again.

Credit must be given to Maire (29 and 30) for having pointed out the resemblance between the nuclear history of the higher plants and that of the Uredineae. He believed, however, that the 'synkaryon' was a special condition always brought about by the association of two daughter-nuclei. He considered the spermogonia and aecidia to be non-sexual in nature, and was ignorant of any process of fertilization such as has just been described for the aecidium of *Phragmidium*.

The existence of actual histological differences between gametophyte and sporophyte is in full agreement with the usual rigid distinction which is to be observed between the two generations, both in heteroecious and autoecious forms. As is well known, the aecidiospore by infection gives rise only to a mycelium bearing uredospores or teleutospores, the uredospore gives rise to a mycelium bearing only uredospores again, or teleutospores. Again, the sporidium arising from the teleutospore gives rise by infection to a mycelium bearing only aecidiospores, if those are present in the life-history (i. e. in the *eu-* and *-opsis* forms)¹.

Apogamy. In the forms in which the aecidium is absent (*brachy-*, *lepto-*, *micro-*, and *hemi-* forms) the sporidium produces a mycelium which gives rise directly to uredospores or teleutospores with paired nuclei. In such a case we have a transition from the sexual to the asexual generation without the intervention of the female reproductive organ, and thus it is clearly comparable to *apogamy* among the higher plants. Sapin-Trouffy has shown that the change from single to paired nuclei takes place in these cases in connexion with the formation of the uredospores, or of the teleutospores, if the former are absent. The exact cytological details have yet to be worked out. It may be that the binucleate condition is brought about by the *interaction of two vegetative cells*, as in the case of apogamy in Ferns investigated by Farmer, Moore, and Digby (14), where they found the nuclei of neighbouring prothallial cells fusing to form sporophytic tissue. A form like *Puccinia Liliacearum*, DC., seems, like some of the Ferns, to be only imperfectly apogamous, for though the aecidia have been described they do not seem to be usually present.

Apospory. The peculiar cycle of development observed regularly in

¹ The very few observed cases of aberrations from the normal life-history, such as aecidiospores giving rise to a mycelium bearing aecidiospores again (A. P. Dietel, Zeit. für Pflanzenkrankheiten, iii, 1893, p. 25; Flora, lxxxi, 1895, p. 394), require further investigation; such cases may possibly be due to a separation of the nuclei of the pair, as in *Endophyllum*.

Endophyllum, in which binucleate aecidiospores, produced in a normal way, behave on germination like teleutospores, the two nuclei separating and uninucleate sporidia being produced (see Maire, 29), is just the opposite to the case discussed above. It is the transition from the asexual to the sexual generation without the intervention of the teleutospore (spore-mother-cell). It can therefore be compared with cases of *apospory* among the higher plants. Maire has described a case in *Endophyllum* in which the aecidiospore germinated in a normal way, but the life-history of the form is not known.

There can be little doubt that the *eu-* and *-opsis* forms which possess the aecidium must be considered as more primitive and the other (apogamous) forms as reduced. The view of Dietel, that the *lepto-* and *micro-* forms which possess only teleutospores are the most primitive, and that the other forms are derived from them, can hardly be accepted in the light of our present knowledge.

Heteroecism. As the heteroecious forms are confined to those possessing the aecidium, i.e. to the more primitive, it seems probable that *heteroecism* may not be, as generally conceived (see Klebahn, 27), a later adaptation, but may actually be the primitive condition in the group. Although we are ignorant of the origin of the group it is possible to conceive that the sporophyte was first developed in connexion with life on another host, just as the sporophyte in the higher plants seems to have been developed in connexion with a new terrestrial existence. The autoecious *eu-* forms would then be the first step in reduction—a purely environmental one; later a morphological reduction of the number of spore-forms would appear to have taken place.

FUSION IN THE BASIDIUM.

The fusion of nuclei in the basidium of the *Basidiomycetes* is exactly comparable to that in the teleutospore, for it has been shown by Maire (30) in the case of a large number of forms, and also by Harper (21) in two cases, that in this group, also, the two nuclei which fuse are conjugate nuclei, the ancestors of which have been associated together for a number of generations. It is clear then that the view of Dangeard and others, which considers this fusion as a process of fertilization, is quite untenable. The stage at which the nuclei first become conjugate is unknown, but it is there that one must look for a process of fertilization, and not to the fusion in the basidium, which, like that in the teleutospore, is a process of mere nuclear reduction, and (like chromosome-reduction) the necessary sequence of fertilization if the sexual cycle is to be completed.

As the *Basidiomycetes* are apparently without anything which could be considered even as a reduced form of sexual organ, it is suggested that the conjugate nuclear condition, the transition from gametophyte to

sporophyte, is brought about in a purely *apogamous* way (as in the case of the Uredineae without an aecidium), perhaps by the interaction of two vegetative cells, as in the union of vegetative cells of the prothallium mentioned above. Maire (30) has stated that the basidiospore gives origin to a mycelium with single nuclei (thus agreeing with the sporidium, which has always been found to behave in this way), and that later in the mycelium the nuclei become paired, but in what manner is not known. The condition of paired nuclei seems in some cases to arise very early, for Harper (21) was unable to observe in *Hypochnus* any but binucleate cells; the germination of the basidiospore, however, was not followed.

The Basidiomycetes would thus seem to resemble very closely the *lepto-* forms among the Uredineae, where the mycelium has at first single nuclei but later develops paired nuclei in connexion with the formation of teleutospores, and like them would appear to have *an alternation of generations obscured, however, by the apogamous transition from one to the other.*

The fusion of nuclei in the basidium, like that in the teleutospore, is followed by a tetrad division, and there seems little doubt that it is also followed by a process of chromosome-reduction, or one corresponding to it; the basidium, like the teleutospore, seems also of the nature of a spore-mother-cell, and is to be compared to that of the higher plants. The reduction in the basidium from four to two chromosomes described by Maire (30) as general for the Basidiomycetes can hardly be accepted, since these structures appear to represent *groups* of chromosomes, but the observations show clearly the analogy with *Gymnosporangium*. Whether the nuclear divisions in the Basidiomycetes will be found sufficiently typical to allow of the observation of an actual numerical reduction remains for future inquiry.

Maire has also described a process of synapsis in the fusion-nucleus of the basidium, comparable to that here described for the teleutospore.

RELATIONSHIPS OF THE UREDINEAE.

It is obvious that the Uredineae and Basidiomycetes are very closely related, for both possess single nuclei during the early part of their life-history (starting from the teleutospore and basidium respectively), which later become paired. In both cases, also, the paired nuclei fuse in special reproductive cells, the fusion being followed by a process of synapsis, and then by a tetrad division¹. The Basidiomycetes seem wanting in all trace of definite sexual organs, so that the Uredineae must be considered as by far the more primitive and cannot be treated as a mere class of the Basidiomycetes, as in the well-known classification of Brefeld. As pointed out in the previous section the latter group appear rather to be reduced, apogamous forms of the Uredineae.

¹ As is well known the transversely divided teleutospore of *Coleosporium* is practically indistinguishable from a transversely divided basidium.

The existence of immotile male cells (spermatia) and of a sterile cell (in *Phragmidium*) which may possibly have formerly had the function of a trichogyne certainly points to a relationship of the Uredineae with the Florideae, a relationship which has been suggested by Meyer (35), chiefly on anatomical grounds, for all the higher Fungi. It is possible that an alternation of generations may yet be found in this algal group, for tetraspore-formation suggests a process of chromosome-reduction; the fact that in the majority of forms there is a distinction of sexual and asexual plants is also very striking in this connexion. Such a discovery—and it must be remembered that Williams (59 and 60) has already observed this alternation in the Dictyotaceae with chromosome reduction in the tetraspore-mother-cell—would certainly lend strong support to a view of a relationship between the Florideae, Uredineae, and Basidiomycetes.

SUMMARY.

The peculiar nuclear cycle described by Sapin-Trouffy for the Uredineae was confirmed in the case of *Phragmidium violaceum*, Wint., and of *Gymnosporangium clavariaeforme*, Rees. In this cycle, the mature teleutospore is uninucleate and gives rise to four uninucleate sporidia, from which a mycelium is developed with the nuclei arranged singly, usually in separate cells. The spermatia produced on this mycelium are uninucleate, but in the young aecidium the nuclei become paired (forming binucleate cells) and divide together in very close association. This paired condition is then persistent throughout the rest of the life-cycle (aecidiospores, uredospores, and mycelia produced from them) up to the formation of the teleutospores, which in the young state are binucleate, but when mature become uninucleate by the fusion of the two paired nuclei. This cycle of development seems common to all the Uredineae (except *Endophyllum*) which have an aecidial stage in their life-history.

A study of the structure of the spermatia of the Uredineae shows that they have the characters not of conidia but of *male cells*, for they exhibit a large dense nucleus, very little cytoplasm, no reserve material, and a very thin cell-wall. These characters, together with their usual association with the aecidia, their absence of function, and the peculiar, apparently reduced, form of fertilization to be observed in the aecidium of *Phragmidium violaceum*, point clearly to the view that the spermatia are male cells which formerly took part in a process of fertilization in connexion with the aecidium, but have now become functionless.

The aecidium of *Phrag. violaceum* is developed immediately beneath the epidermis of the leaf and has a very simple structure. It consists of a layer of special cells, each of which cuts off a *sterile cell* above, which soon disorganizes, while the lower, the *fertile cell*, increases in size and shows abundant protoplasm, and after a pause in its development is *fertilized by*

the migration into it of the nucleus of one of the undifferentiated mycelial cells at its base. It then undergoes a series of rapid divisions, cutting off a series of aecidiospore-mother-cells. The 'fertile cell' has thus the characters of a *female cell*.

The aecidium of *Phrag. violaceum* is to be considered as a *sorus of female reproductive organs*, each of which consists of a *sterile cell* above and a *female cell* below, the nucleus of an ordinary vegetative cell bringing about fertilization and performing the part which was apparently formerly taken by the nucleus of the spermatium. The female cell thus develops by means of a *reduced form of fertilization*.

It is suggested that the sterile cell is reduced, and that it formerly pushed its way to the surface (as it can sometimes now be observed to do) and acted as a *trichogyne* to bring the spermatium into relation with the female cell below.

In the process of fertilization the two nuclei do not fuse, but merely remain very closely associated (as in the egg of *Cyclops*); there is thus started in the female cell the condition of paired nuclei which continues up to the teleutospore.

The aecidium throughout the group must be considered as a *sorus of reduced female reproductive organs*, for it appears to be always the fertile cell which becomes binucleate. Whether in all cases there is a nuclear migration, or whether in some there is a still further reduction and the process consists of an association of two daughter-nuclei, has yet to be determined, as has also the question of the presence or absence of a sterile cell.

As the spermogonia and aecidia are to be considered as male and female reproductive organs, respectively, it is evident that the Uredineae which possess an aecidial stage in their life-history (*eu-* and *-opsis* forms) exhibit a well-marked *alternation of generations* which are not only to be distinguished as sexual and asexual, but are also to be sharply differentiated cytologically, the *sexual* generation being characterized by single nuclei (with two chromatin masses on division), the *asexual* by paired nuclei (with four chromatin masses on division). The transition from the gametophyte to the sporophyte takes place in the aecidium and the transition from the sporophyte to the gametophyte in the teleutospore. The alternation of generations is thus as clearly marked as that of the higher plants or of the Dictyotaceae.

The fusion in the teleutospore of the two nuclei—the direct descendants of those which first became associated in the *fertile (female)* cell of the aecidium—is clearly not in itself a process of fertilization (nor the teleutospore an egg-cell), as Dangeard and Sapin-Trouffy supposed, but a mere secondary process, the result of fertilization and the preliminary to reduction. It is pointed out that three nuclear stages are to be observed in the sexual cycle of plants and animals—*nuclear association*, *nuclear reduction* (so-called *fusion*), and *chromosome-reduction*. Of these three stages, only the first

is the essential part of fertilization (as various plants and animal eggs and that of *Cyclops* show) ; the second may take place at the same time as the first, or it may be delayed for a time, or, as in the Uredineae, it may be delayed until the stage corresponding to chromosome-reduction.

The fusion-nucleus in the teleutospore undergoes changes which correspond to *synapsis*, and when it divides there is seen to be a reduction from the four chromatin masses of the paired nuclei to two chromatin masses ; owing to the absence of chromosome-formation in most of the nuclear divisions an actual reduction in number of chromosomes cannot be observed. The process of fusion and reduction in the teleutospore is followed by a definite tetrad division in the 'promycelium,' so that the teleutospore corresponds exactly with the spore-mother-cell of the higher plants, the 'promycelial' cells being really of the nature of spores.

As the fusion of the two nuclei is delayed throughout the whole of the sporophyte there is associated with the reduction from four to two chromatin masses a process of nuclear reduction from two nuclei to one.

Nuclear division in most of the cells of the Uredineae is of an exceedingly simple type. The nuclei (whether single or paired) lose their membrane, the nucleolus becomes extruded, and the chromatin condensed into one, or sometimes two, masses. A rudimentary spindle can sometimes be observed, and upon this the chromatin becomes spread and is drawn apart into two or four pear-shaped masses which separate and form the daughter-nuclei. In the paired (conjugate) state the nuclei divide side by side in close juxtaposition, passing *pari passu* through the various stages of division, as described by earlier observers.

In the promycelium the two divisions are much more typical. In *G. clavariaeforme* a well-marked spindle with centrosomes and polar radiations was observed. The first division showed a formation of numerous chromosomes, though their behaviour on the spindle is not typical and there is doubt as to whether a definite splitting actually occurs. In the second division the chromatin forms no chromosomes, but merely a network which covers the spindle, and is later drawn apart into two portions.

The spindle in the case of the second division in the promycelium of *G. clavariaeforme*, and apparently also in the case of the first, is formed free in the cytoplasm between the two portions of a divided centrosome (like the 'Centralspindel' of Hermann), and afterwards comes into close relation with the nucleus.

The simple form of division found in the Uredineae is to be considered as reduced from the typical method of karyokinesis.

The two structures observed constantly during nuclear division by Sapin-Trouffy and Maire, on which they base their views of chromosome-reduction, cannot be considered of the nature of chromosomes, but are merely chromatin masses. They probably represent the chromatin derived

respectively from the two nuclei of the teleutospore, but are not always present. In the first division of the promycelium of *G. clavariaeforme* they can be seen to be formed by the aggregation of a number of chromosomes; in the other divisions they are formed directly, by the condensation of the chromatin of the nucleus.

In those Uredineae which have a reduced life-cycle without an aecidium (*brachy*-, *hemi*-, *micro*-, and *lepto*- forms) the transition from single to paired nuclei, and from gametophyte to sporophyte, apparently takes place in connexion with the uredospores, or if these are absent, in connexion with the teleutospores. Such a shortening of the life-cycle is comparable with the case of *apogamy* among the higher plants; the cytological details are not yet known, but it is possible that it is brought about by the association of the nuclei of two vegetative cells (cf. *apogamy* in ferns).

The peculiar shortening of the life-cycle to be observed in *Endophyllum*, where the binucleate aecidiospore germinates like a teleutospore and the uninucleate condition is brought about by the simple separation of the two nuclei, is clearly a transition from the sporophyte to the gametophyte without the intervention of the teleutospore (spore-mother-cell), and should accordingly be considered as comparable to *apospory* in the higher plants.

The fusion of paired nuclei in the *basidium* of the Basidiomycetes is exactly comparable with that in the teleutospore, and should also be considered, not as a process of fertilization, but as a purely secondary process of nuclear reduction preliminary to chromosome reduction.

How the condition of paired nuclei is brought about in the Basidiomycetes is not yet clear. The life-history of these forms seems comparable with that of the *lepto*- forms among the Uredineae, and it is suggested that, in the absence of sexual organs, the transition from single to paired nuclei, i.e. the transition from gametophyte to sporophyte, is brought about *apogamously* in a similar way.

The Uredineae and Basidiomycetes show an obvious relationship, for both have paired nuclei at some stage of their life-history, and in both groups the individuals of certain pairs fuse together in specialized reproductive cells, a fusion to be followed by synapsis and a tetrad division. The Uredineae with their sexual organs would certainly seem to be more primitive and can hardly be classed as a mere subdivision of the Basidiomycetes.

The Uredineae appear to show a relationship with the Florideae among the Algae.

The aecidium of *Phragmidium* with superficial position and very simple structure without a pseudoperidium is really not a definite organ, but a mere ill-defined sorus of reduced, female reproductive organs; it is no doubt *primitive*, while the typical aecidium with its pseudoperidium and deeper point of origin is a definite organ, and is probably a later development.

The binucleate condition of the sporidium exhibited by *Phrag.*

violaceum and some other forms is without particular significance, and is apparently a mere precocious division in which wall-formation is delayed. The view which has been put forward, that the sporidium starts in the life-history the condition with paired nuclei, is without foundation, for in all cases observed the mycelium arising from the sporidium has single nuclei, even in such cases as *Phrag. violaceum*, where the sporidium is known to be usually binucleate.

The teleutospore is really of the nature of a spore-mother-cell, the contents of which, usually after germination (but directly in *Coleosporium*), break up by a tetrad division into a series of four primary spores (the so-called promycelial cells). These can separate and put out germ-tubes, and no doubt cause infection; normally, however, when growing in free air, they remain connected and their germ-tubes become arrested, so that four secondary spores—the sporidia—are formed. The term promycelium is thus a misnomer.

NOTE.

It is obvious that the method of development of the fertile (female) cells in the aecidium of *Phragmidium* may be considered as a new type of so-called *parthenogenesis*; for, just as in *Artemia* there is a fertilization of 'parthenogenetic' eggs by the second polar body (nucleus) instead of by a spermatozoon, so in this case the nucleus of a vegetative cell has apparently replaced in function that of the spermatium. The term parthenogenesis is, however, an unsatisfactory one, as it is not very aptly applied to these cases in which there is a process of nuclear fusion; it would be much more satisfactory to confine the term to cases in which the sexual cells actually develop with the reduced number of chromosomes, without any form of fertilization. There is the same difficulty with *apogamy* now that we know that in apogamous Ferns the nuclei of prothallial cells may fuse together, for the application of this term suggests a denial of the existence of any process of fusion of cells or nuclei which could be considered of the nature of gametic fusion.

It becomes clear that cytological investigations of recent times have practically broken down the distinction between fertilization, parthenogenesis (in the wide, but not in the narrow, sense suggested above), and apogamy. Between the fusion of an egg-cell with a differentiated male cell, the fusion (association) of an egg-cell with a vegetative cell (or nucleus), the fusion of an egg-cell and its polar body, and the fusion of two vegetative cells (or nuclei) no sharp line can be drawn, and they must all be considered as terms in a series of *fertilizations*. Just as on one side of normal oogamous fertilization with its differentiated male and female cells there are such primitive types of fertilization as isogamy, and that, the most primitive, in which there is no distinction of sexual and vegetative cells; so, on the other side, there is a series of *reduced* processes of gradually increasing simplicity, the most simple being practically a return to the most primitive, where the sexual and vegetative cells are alike. Apart from the presence of the functionless spermata and the peculiar migration of the nucleus through a cell-wall, which show that it is reduced in evolution, there is nothing to clearly distinguish the process observed in *Phragmidium* from such accepted fertilizations as the conjugation of adjacent cells in a *Spirogyra* filament, the fusion of gametes (which are of the relationship of cousins) in *Actinosphaerium*, or from such a process as is to be observed in *Basidiobolus*, or even other Fungi, in which the sexual organs arise on neighbouring cells of a hypha.

LIST OF PAPERS.

1. ARTHUR, J. C. : Problems in the Study of Plant Rusts. Bull. Torrey Bot. Club, xxx, 1903, p. 1.
2. ————— : The Aecidium as a Device to restore Vigour to the Fungus. Soc. prom. Agric. Sci. 23rd Ann. Meeting, 1903 (not seen).
3. DE BARY, A. : Untersuchungen über die Brandpilze. Berlin, 1853.
4. ————— : Vergleichende Morphologie u. Biologie der Pilze u.s.w. Leipzig, 1884.
5. BAUR, E. : Zur Frage nach der Sexualität der Collemaceae. Ber. d. deutsch. Bot. Ges., xvi, 1898, p. 363.
6. BLACKMAN, V. H. : On the Cytological Features of Fertilization and Related Phenomena in *Pinus silvestris*, L. Phil. Trans. Roy. Soc., cxc, 1898, p. 395.
7. ————— : On the Conditions of Teleutospore-germination and Sporidia-formation in the Uredineae. New Phytologist, ii, 1903, p. 10.
8. ————— : On a new Method for facilitating the Staining of microscopically small Objects. Ibid., ii, 1903, p. 103.
9. BOVERI, TH. : Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh. Phys.-Med. Ges. Würzburg, xxxv, 1902, p. 67.
10. BREFELD, O. : Untersuch. aus dem Gesamtgebiete der Mykologie, vii, 1888, p. 60; viii, 1889, p. 230; ix, 1891, p. 26.
11. CONKLIN, E. G. : Karyokinesis and Cytokinesis in the maturation, &c., of *Crepidula*. Journ. Acad. Philad., xii, Pt. I, 1902.
12. CORNU, M. : Étude de la fécondation dans la classe des Champignons. Compt. Rend., Juin, 1875, p. 1466.
13. DANGEARD, P. A., and SAPIN-THOUFFY, P. : Une pseudofécondation chez les Uredinées. Compt. Rend., cxvi, 1893, p. 267.
14. FARMER, J. B., MOORE, J. E., and DIGBY, L. : On the Cytology of Apogamy and Apospory. I. : Prelim. note on Apogamy. Proc. Roy. Soc., lxxi, 1903, p. 453.
15. ————— and MOORE, J. E. S. : New Investigation into the Reduction Phenomena of Animals and Plants. Proc. Roy. Soc., lxxii, 1903, p. 104.
16. FERGUSON, M. C. : The Development of the Egg and Fertilization in *Pinus Strobus*. Annals of Botany, xv, 1901, p. 435.
17. HÄCKER, V. : Ueber die Selbständigkeit der väterlichen und mütterlichen Kernbestandtheile u.s.w. Archiv für mikr. Anat., xlvi, 1895, p. 579.
18. ————— : Die Keimbahn von Cyclops. Archiv für mikr. Anat., xlix, 1897, p. 35.
19. ————— : Mitosen im Gefolge amitosenähnlicher Vorgänge. Anat. Anzeig., xvii, 1900, p. 9.
20. HARPER, R. : Sexual reproduction in *Pyronema confluens*, &c. Annals of Botany, xiv, 1900, p. 321.
21. ————— : Binucleate Cells in certain Hymenomycetes. Bot. Gaz., xxxiii, 1902, p. 1.
22. HEDLUND, T. : Ueber Thallusbildung durch Pyknokonidien bei *Catillaria denigrata*, Fr. u. *C. prasina*, Fr., Bot. Centralbl., lxiii, 1895, p. 9.
23. HOLDEN, R. J. and HARPER, R. A. : Nuclear Division and Nuclear Fusion in *Coleosporium Sonchi-arvensis*, Lev. Trans. Wisconsin Acad. Sci., &c., xiv, 1903, p. 63.
24. ISTVÁNYFI, G. : Ueber die Rolle der Zellkerne bei der Entwicklung der Pilze. Ber. d. deutsch. Bot. Ges., xiii, 1895, p. 459.
25. JUEL, H. O. : Die Kerntheilungen in den Basidien und die Phylogenie der Basidiomyceten. Jahrb. für wiss. Bot., xxxii, 1898, p. 361.
26. KLEBAHN, H. : Kulturversuche mit heteröcischen Uredineen. Zeitschr. für Pflanzenkrank. ii, 1892, p. 264; ibid. v, 1895, p. 69.
27. ————— : Die wirtswechselnden Rostpilze. Berlin, 1903 (dated 1904).
28. LAUTERBORN, R. : Untersuch. über Bau, Kernteilung u. Bewegung der Diatomeen. Leipzig, 1890.
29. MAIRE, R. : L'évolution nucléaire chez les *Endophyllum*. Journ. de Bot., xiv, 1900, p. 80.
30. ————— : Recherches cytologiques et taxonomiques sur les Basidiomycètes. Bull. Soc. Mycol. de France, xviii, 1903, pp. 1-209.

31. MCGREGOR, J. H.: The Spermatogenesis of Amphiuma. *Journal of Morphology*, xv, 1899 (Supplement), p. 57.
32. MEEVES, FR.: Über amitotische Kernteilungen in den Spermatogonien des Salamanders u. s. w. *Anat. Anzeig.*, vi, 1891, p. 626.
33. MEZGER, O.: Untersuch. über die Entwicklung der Flechtenfrucht. *Beitr. zur wiss. Bot.*, v, 1903, p. 108.
34. MEYEN, F. J. F.: *Pflanzen-Pathologie*. Berlin, 1841, p. 143.
35. MEYER, A.: Die Plasmaverbindungen und die Fusionen der Pilze der Florideenreihe. *Bot. Zeit.*, lx, 1902, p. 139.
36. MÖLLER, A.: Über die Cultur flechtenbildender Ascomyceten ohne Algen. *Untersuch. aus dem Forst-Bot. Inst. Münster*, 1887.
37. MONTGOMERY, T. H.: A Study of the Chromosomes of the Germ-cells of the Metazoa. *Trans. Amer. Phil. Soc.*, xx, 1901, p. 154.
38. —————: The Heterotypic Maturation Mitosis in Amphibia and its general significance. *Biol. Bull.*, iv, 1903, p. 258.
39. NATHANSOHN, A.: Physiologische Untersuchungen über amitotische Kerntheilung. *Jahrb. f. wiss. Bot.*, xxxv, 1900, p. 48.
40. NĚMEC, B.: Ueber die Einwirkung des Chloralhydrats auf die Kern- und Zellteilung. *Jahrb. f. wiss. Bot.*, xxxvii, 1902, p. 8.
41. OVERTON: Mikrotechnische Mitteilungen. *Zeitschr. f. wiss. Mikrosk.*, vii, 1890, p. 9.
42. PLOWRIGHT, C. B.: *British Uredineae and Ustilagineae*. London, 1889.
43. POIRAULT, G., and RAČIBORSKI, M.: Sur les noyaux des Urédinées. *Journ. de Bot.*, ix, 1895, p. 318.
44. RAČIBORSKI, M.: Ueber den Einfluss äusserer Bedingungen auf die Wachstumsweise des *Basidiobolus ranarum*. *Flora*, lxxxii, 1896, p. 107.
45. ROSEN, F.: Beiträge zur Kenntniss der Pflanzenzellen. *Beitr. zur Biol. der Pflanzen*, vi, 1892, p. 255.
46. RÜCKERT, J.: Ueber das Selbständigbleiben der väterlichen und mütterlichen Kernsubstanz u. s. w. *Archiv für mikr. Anat.*, xlv, 1895, p. 3.
47. RUHLAND, W.: Ueber intracelluläre Karyogamie bei den Basidiomyceten. *Bot. Zeit.*, lix, 1901, p. 187.
48. SAPIN-TROUFFY, P.: Recherches histologiques sur la famille des Urédinées. *Le Botaniste*, 5^e série, 1896, p. 59.
49. SCHMITZ, F.: Ueber die Zellkerne der Thallophyten. *Verh. naturhist. Vereins d. preuss. Rheinlande u. Westfalens*, xxxvii, 1880, p. 195.
50. SHIBATA, K.: Cytologische Studien über endotrophe Mykorrhizen. *Jahrb. f. wiss. Bot.*, xxxvii, 1902, p. 643.
51. SUTTON, W. S.: On the Morphology of the Chromosome Group in *Brachystola magna*. *Biol. Bull.*, iv, 1903, p. 26.
52. —————: The Chromosomes in Heredity. *Biol. Bull.*, iv, 1903, p. 231.
53. TULASNE, L. R.: Second mémoire sur les Urédinées et les Ustilaginées. *Ann. Sci. Nat.*, iv, 2^e sér., 1854, p. 77.
54. VAN TIEGHEM, PH.: Sur la classification des Basidiomycètes. *Journ. de Bot.*, vii, 1893, p. 77.
55. WAGER, H.: On Nuclear Division in the Hymenomycetes. *Annals of Botany*, vii, 1893, p. 489.
56. —————: On the Presence of Centrospheres in Fungi. *Ibid.*, viii, 1894, p. 321.
57. —————: The Sexuality of the Fungi. *Ibid.*, xiii, 1899, p. 575.
58. WASIELEWSKI, W.: Theoretische und experimentelle Beiträge zur Kenntniss der Amitose. *Jahrb. f. wiss. Bot.*, xxxvii, 1902, p. 8.
59. WILLIAMS, J. L.: Alternation of Generations in the Dictyotaceae. *New Phytologist*, ii, 1903, p. 184.
60. —————: Studies in the Dictyotaceae, I. *Annals of Botany*, xviii, 1904, p. 146.
61. ARTHUR, J. C.: Taxonomic Importance of Spermogonia. *Bull. Torrey Bot. Club*, xxxi, 1904, p. 113.

EXPLANATION OF PLATES

Illustrating Mr. V. H. Blackman's paper on the *Uredineae*.

PLATE XXI.

Figs. 1-13. *Phragmidium violaceum*, Wint.

Fig. 1. Teleutospore, lying in water, showing general form and stalk with swollen base; the two upper cells have just begun to germinate. $\times 450$.

Fig. 2. Mature teleutospore, taken in autumn, in longitudinal section, showing the single nuclei and the structure of the wall and pits; the uppermost cell is not cut exactly through the middle, so the nucleus and the apical peg of thickening are not shown. $\times 800$.

Fig. 3. Nucleus of the cell of a teleutospore, gathered in spring, which had been lying in water twelve hours. $\times 1900$.

Fig. 3a. Similar nucleus, but showing a later stage with single spireme thread. $\times 1900$.

Fig. 4. Transverse section of cell of teleutospore, showing vacuolate nucleus; wall structure not shown. $\times 1150$.

Fig. 4a. Similar section, but showing abnormal state with *two* nuclei in the cell. $\times 1150$.

Fig. 5. Teleutospore in optical longitudinal section, showing development of long germ-tube; the apical cell has already germinated and is empty; the nuclei in the two lower cells appear as clear areas. Fresh preparation. $\times 450$.

Fig. 6. Germinating cell of teleutospore in transverse section, showing passage of tube through pit. $\times 970$.

Fig. 7. End of germ-tube (promycelium) with nucleus. $\times 600$.

Fig. 8. Promycelium divided into four cells, each of which has put out a germ-tube; one nucleus has migrated into the tube. $\times 1150$.

Fig. 8a. Promycelium with three sporidia and one cell not yet germinated. $\times 1150$.

Fig. 9. Mature promycelium from which two of the sporidia have fallen; one of the two remaining is germinating *in situ*. $\times 950$.

Fig. 10. Primary sporidium in section with single nucleus. $\times 1350$.

Fig. 11. Secondary sporidium in section with two nuclei. $\times 1350$.

Fig. 12. Primary sporidium, still attached to sterigma, showing two nuclei. $\times 1350$.

Fig. 13. Secondary sporidium developed from primary and showing, apparently, four nuclei. $\times 1350$.

Figs. 14-35. *Gymnosporangium clavariaeforme*, Rees.

Fig. 14. Mature two-celled teleutospore about to germinate. $\times 650$.

Fig. 15. Cell of similar teleutospore in transverse section. $\times 1350$.

Fig. 16. Germinated teleutospore, showing nucleus moving into tube on left, and nucleus dividing in middle of tube on right. $\times 620$.

Fig. 17. Early prophase of division of teleutospore nucleus in germ-tube (promycelium), showing centrosome and kinoplasm. $\times 1900$.

Fig. 18. Prophase showing chromatin collected, apparently as a single thread, towards one end of nucleus. $\times 1900$.

Fig. 19a and b. Two consecutive sections through a germ-tube with dividing nucleus; the majority of the chromosomes are to be found in a, while b shows the very young spindle with centrosomes. $\times 1900$.

Fig. 20. Chromosomes collected irregularly round spindle; one pole shows apparently two centrosomes. $\times 1900$.

Fig. 21. Chromosomes gathered towards centre of spindle. $\times 1900$.

Fig. 22. Similar stage to above, but spindle oblique in position. $\times 1900$.

Fig. 23. Chromosomes beginning to spread out on spindle; distinct radiations from upper pole. $\times 1900$.

Fig. 24. Chromosomes are shorter and show a tendency to arrange themselves in two rows and apparently to fuse together. $\times 1900$.

Fig. 25. Metaphase with shortened chromosomes stretched out on spindle. $\times 1900$.

- Fig. 25 *a*. Polar view of chromosomes on spindle. $\times 1900$.
 Fig. 26. Anaphase showing chromosomes separating into two groups. $\times 1900$.
 Fig. 27. Similar stage to above, but each group forms apparently a chromatin network. $\times 1900$.
 Fig. 28. Anaphase showing *two* groups of chromosomes moving towards each pole. $\times 1900$.
 Fig. 29. Later stage, showing chromosomes continuous from pole to pole and partially fused. $\times 1900$.
 Fig. 30. Later stage, showing two distinct chromatin masses continuous from pole to pole. $\times 1900$.
 Fig. 31. Chromatin forms an irregular mass at each pole with remains of elongated spindle between them; the radiations from the upper centrosome were about twice as long as shown and extended to the free end of the germ-tube. $\times 1900$.
 Fig. 32. Similar stage to above, but two distinct chromatin masses at one pole. $\times 1900$.
 Fig. 33. Daughter-nuclei formed; each shows kinoplasmic material (remains of spindle) and a centrosome with radiations. $\times 1050$.
 Fig. 34. First stage of second division; nucleus irregular and shows two centrosomes. $\times 1900$.
 Fig. 34 *a*. Early stage of second division in promycelium, showing spindle in young state, lying on one side of nucleus. $\times 1900$.
 Fig. 35. Later stage, in which spindle is now arranged axially in the tube and is partly surrounded by the chromatin mass. $\times 1900$.

PLATE XXII.

- Figs. 36-45. *G. clavariaeforme*.
 Fig. 36. End of promycelial tube, showing second division with spindle now lying in the centre of the chromatin mass. $\times 1900$.
 Fig. 37. Section through spindle, showing chromatin in the form of granules. $\times 1900$.
 Fig. 38. Tube showing the two nuclei in different stages of division. At *a* the chromatin forms a distinct network and covers three-fourths of the spindle; at *b* a later stage is visible, in which the spindle is completely hidden by the elongated chromatin network, and only the centrosomes with their radiations are to be seen. $\times 1900$.
 Fig. 39. The two main portions of the chromatin network have drawn apart towards the poles, but are still connected by a number of threads. $\times 1900$.
 Fig. 40. Chromatin portions completely separated at the poles; the projecting arms are the remains of the threads which connected the portions in an earlier stage, as in Fig. 39. $\times 1900$.
 Fig. 41. A somewhat later stage than above; the projecting arms have been drawn in, and the chromatin forms a single mass at each pole.
 Fig. 41 *a*. A similar stage to above, but chromatin forms *two* distinct masses at one pole. $\times 1900$.
 Fig. 42. Almost complete germ-tube (promycelium), showing the nuclei just formed, and a transverse wall in middle. In three of the nuclei the centrosome and the small portion of kinoplasm which connects it with the nucleus are clearly visible. $\times 1900$.
 Fig. 43. The four cells of the promycelium are formed. Three of the nuclei show a chromatin network, the other is in a younger state and still remains almost homogeneous. $\times 1350$.
 Fig. 44. Promycelium with outgrowths from each cell. The outgrowths are of the nature of germ-tubes rather than of sterigmata. $\times 880$.
 Fig. 45. Sporidium. $\times 1350$.
 Fig. 46. Portion of spermogonium of *Phrag. violaceum* on leaf of *Rubus*, showing general characters and thickened ruptured cuticle. $\times 660$.
 Fig. 47. Group of spermatia of same. $\times 1900$.
 Figs. 48-59. *G. clavariaeforme*.
 Fig. 48. Section through spermogonium on leaf of *Crataegus*. The spermatial hyphae, spermatia, paraphyses, basal tissue (plectenchyme), and host tissue are all clearly visible. $\times 520$.
 Fig. 49-54. Stages in the development of a spermatium on a spermatial hypha. $\times 1350$.
 Fig. 55. Dividing nucleus of spermatial hypha, showing chromatin collected into a solid mass. $\times 1900$.
 Fig. 55 *a*. Stage showing nucleolus being expelled as chromatin shrinks to a homogeneous mass. $\times 1900$.

Fig. 56. Later stage than above, showing chromatin separating into two daughter-masses. $\times 1900$.

Fig. 57. Division in spermatial hypha in which chromatin shows a longitudinal division into two masses. $\times 1900$.

Fig. 58. Very young spermatia with chromatin still an almost solid mass. $\times 1900$.

Fig. 59. Group of mature spermatia. $\times 1900$.

Figs. 60-65. Aecidium-development in *Phrag. violaceum*.

Fig. 60. Peripheral portion of young aecidium, showing the simplicity of structure. On the extreme left the special cells developed beneath the epidermis of the leaf are still undivided, while towards the middle a distinction into *fertile* and *sterile* cells can be observed and some of the fertile cells have already become binucleate. On the right the division of the fertile cells and the formation of aecidiospores and intercalary cells can be seen; the epidermis at this part has been ruptured by the development of the aecidiospores. $\times 340$.

Fig. 61. Small portion of young aecidium, showing epidermal cells of leaf, sterile cells and fertile cells. Two of the fertile cells have become binucleate and one is still uninucleate; the one on the right has begun to grow up and push through the sterile cell. $\times 1350$.

Fig. 62. Fertile cell with two nuclei of dissimilar structure. $\times 1350$.

Fig. 63. Portion of aecidium showing fertile cell with nuclei of unequal size and different structure; the nuclei of the sterile cells have become partly disorganized. $\times 1350$.

Fig. 64. Similar to above. $\times 1350$.

Fig. 65. Fertile cell showing small densely staining nucleus in contact with larger nucleus with well-marked nucleolus. $\times 1350$.

PLATE XXIII.

Figs. 66-77 *a*. Aecidium-development of *Phrag. violaceum*, continued.

Fig. 66. Portion of young aecidium, showing nucleus of cell (basal cell) below one fertile cell migrating into another fertile cell. Epidermis of leaf seen above and mesophyll below. $\times 1350$.

Fig. 67. Similar stage to above, but sterile cells are empty. $\times 1350$.

Fig. 68. Nucleus of basal cell migrating into fertile cell immediately above. $\times 1350$.

Fig. 69. Very early stage of migration. $\times 1350$.

Fig. 70. Late stage of migration; the distinction between the two nuclei very clear. $\times 1350$.

Fig. 71. Fertile cell which has cut off the first aecidiospore-mother-cell. $\times 1050$.

Fig. 72. Division of paired nuclei (conjugate division) in the first cell cut off from the fertile cell. $\times 1050$.

Fig. 73. Later stage, in which the nuclei of the one aecidiospore and the intercalary cell are constituted, but no wall is yet formed between them. $\times 1050$.

Fig. 74. Cell-row of aecidium showing, from above downwards, young aecidiospore, intercalary cell, aecidiospore-mother-cell, and fertile cell. $\times 1050$.

Fig. 75. Mature aecidiospore. $\times 1050$.

Fig. 76. Abnormal trinucleate fertile cell which has cut off a trinucleate aecidiospore-mother-cell. $\times 1050$.

Fig. 77. Fertile cell with three nuclei undergoing triple conjugate division; the three solid masses of chromatin and the three nucleoli are to be seen. $\times 1050$.

Fig. 77 *a*. Sterile cell which has grown up between two epidermal cells. $\times 1350$.

Figs. 78-84. Uredospore-development of *Phrag. violaceum*.

Fig. 78. Group of developing uredospores on perennial mycelium, from stem of *Rubus*; four basal cells are marked $\times$. $\times 600$.

Fig. 79. Group of basal cells from which the stalked uredospores will be developed. $\times 1000$.

Fig. 80. Basal cell with young uredospore-mother-cell. $\times 1000$.

Fig. 81. Young uredospore-mother-cell. $\times 1350$.

Fig. 82. Uredospore-mother-cell dividing to form uredospore and stalk-cell. $\times 1350$.

Fig. 83. Young uredospore with stalk-cell. $\times 1350$.

Fig. 83 *a*. Portion of uredospore and stalk-cell to show protoplasmic continuity between the two. $\times 1350$.

Fig. 84. Mature uredospore, showing the two nuclei of an irregular shape. $\times 800$.

Figs. 85-87. Teleutospore development in *Phrag. violaceum*.

Fig. 85. Portion of leaf of *Rubus*, showing young teleutospore-sorus and mycelium; teleutospores in various stages and a few paraphyses are to be seen. Two basal cells of the teleutospores are marked $\times$, and two binucleate haustoria marked *. $\times 500$.

Fig. 86. Three-celled teleutospore with stalk, in very young state. $\times 1350$.

Fig. 86 *a*. Slightly older stage than above; the lowest cell is dividing and separating the fourth cell from the stalk. The wall shows the beginning of the characteristic thickening; the second nucleus in each of the two upper cells has been cut away. $\times 1350$.

Fig. 86 *b*. Uppermost cell of young teleutospore, showing the apical projection only partially obliterated by thickening. $\times 800$.

Fig. 86 *c*. Base of stalk of mature teleutospore, showing its attachment to the basal cell. $\times 1050$.

Fig. 87 *a-c*. Stages in the fusion of the two nuclei in the teleutospore. *a* the nuclei in contact but not yet fused; *b* the nuclei have fused but not the nucleoli; *c* the two nucleoli have fused. $\times 1900$.

PLATE XXIV.

Fig. 88 *a, b*. Further stages in the development of the teleutospore nucleus of *Phrag. violaceum*. *a* a very distinct twisted chromatin thread in the nucleus; *b* nucleus larger and returning to the condition of a simple network. $\times 1900$.

Figs. 89-92. *G. clavariaeforme*.

Fig. 89. Two basal cells showing the development of the outgrowths which give origin to the stalked teleutospores. On the right the basal cell has just put out a second outgrowth, the first having developed into a teleutospore of which only the lower part of the stalk is seen. $\times 1050$.

Fig. 90. Young outgrowth from basal cell, not yet divided. $\times 1050$.

Fig. 91 *a, b, c*. Stages in the fusion of the nuclei in the teleutospore. At *a* the nuclei in contact but not yet fused; at *b* the two nuclei partly fused; at *c* the nuclei fused, but not the nucleoli. $\times 1350$.

Fig. 92. A later stage than above, in which the two nucleoli have fused to a single nucleolus and the chromatin is in the form of a single twisted thread. $\times 1050$.

Fig. 93 *a-e*. Stages of division of paired nuclei from the young dividing teleutospore of *Phrag. violaceum*. $\times 1900$.

Fig. 94. Dividing cell of teleutospore in which each nucleus shows *two* chromatin masses. $\times 1900$.

Fig. 95 *a-d*. Stages of 'conjugate division' from the uredospore-mother-cell of *Phrag. violaceum*. $\times 1900$.

Fig. 96. Similar stage to that of 95 *b*, but the chromatin of each nucleus forms *two* masses. $\times 1900$.

Fig. 97. Uredospore-mother-cell of *Phrag. violaceum* dividing; each of the daughter-nuclei shows *two* chromatin masses. $\times 1900$.

Fig. 98. Conjugate nuclear division, showing four chromatin masses, from developing teleutospore of *G. clavariaeforme*. $\times 1900$.

Fig. 98 *a*. First stage of division; the nuclear membrane has disappeared and the chromatin become condensed: from teleutospore as above. $\times 1900$.

Fig. 99. Haustorium, showing two nuclei, from uredospore-bearing mycelium of *Phrag. violaceum*. $\times 1050$.

Fig. 100. Nuclear division in one of the four promycelial cells of *G. clavariaeforme*. A distinct spindle is visible but no centrosomes; the arrangement of the chromatin is not clear. $\times 1900$.

Higley et Blackman del.

BLACKMAN

Hughley & Blackman del.

Hughley lith. et imp.

Highley et Blackman del.

BLACKMAN

Highley lith. et imp.

Highley et Blackman del.

Highley lith et imp.

Highley et Blackman del.

Highley lith. et imp.

Blackman, Vernon H. 1904. "On the fertilization, alternation of generations, and general cytology of the Uredineae." *Annals of botany* 18, 323–373.
<https://doi.org/10.1093/oxfordjournals.aob.a088968>.

View This Item Online: <https://www.biodiversitylibrary.org/item/234469>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a088968>

Permalink: <https://www.biodiversitylibrary.org/partpdf/318765>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.