The Sexuality of the Fungi[1].

BY

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AMONG the numerous remarkable and interesting observations which have, in recent years, been made upon the cytology of the lower plants, those which throw light upon the sexual processes in the Fungi take a prominent place. Fifteen years ago[2] we knew that the Phycomycetes as a group were sexual, that the Ascomycetes, according to one school, exhibited phenomena which could be regarded as sexual, but that the other groups of Fungi exhibited no sexual features at all, unless certain cell-unions occurring in some forms could be regarded as such. We were practically ignorant of their cytology, and even the presence of nuclei was regarded by many observers as doubtful.

Now we are not only acquainted with the minute details of nuclear and cell division in a number of forms belonging to the different groups, but the cytological features of fertilization in the Phycomycetes have been investigated and the phenomena brought into line with those occurring in the higher plants and animals, so that we may say of this group, as of the higher plants and animals, that 'the act of fertilization

[1] Afternoon lecture delivered before the Botanical Section at the Dover meeting of the British Association, 1899.

[2] See Marshall Ward, on the Sexuality of the Fungi, Q. J. M. S., vol. xxiv, 1884, for an excellent account of what was known at that time.

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consists in the definite fusion of a male nucleus with a female nucleus to form the primary nucleus of the embryo or new generation.'

In the higher groups (the Ustilagineae, Uredineae, Ascomycetes, and Basidiomycetes) the question of their sexuality has recently been brought prominently forward by the discovery that in certain of their cells a fusion of nuclei takes place at a period just antecedent to, or during, the formation of spores, which is regarded by some observers as a true sexual process, and which in some respects certainly does appear to take the place of, and produces the same results as, the ordinary phenomena of sexuality.

In the following pages I propose to deal first of all with the phenomena of fertilization occurring in the lower Fungi, secondly with the nuclear fusions in the higher Fungi, and thirdly with some theoretical considerations concerning the phenomena of sexuality and nuclear fusion in the whole group.

Phycomycetes.

The Peronosporeae[1] exhibit a distinct sexual differentiation in the formation of male and female elements in distinct cells, oogonia (ooangia) and antheridia. The oogonium is produced as a terminal or intercalary swelling on a hyphal filament inside the host-plant. The antheridium is a tubular outgrowth which may be formed on the same filament as the oogonium or on another one in its immediate neighbourhood. Both contain protoplasm and numerous nuclei. The antheridium comes into contact with the oogonium, and curves closely around it. The nuclei of the oogonium, and probably of the antheridium, then divide by a process of karyokinesis each into two, so that there are now twice as many. The contents of the oogonium separate into two distinct parts—a peripheral portion (the periplasm), which contains all the nuclei, and a central spherical portion (the gonoplasm), which

[1] Wager, Ann. Bot., iv, 1889, and x, 1896; Berlese, 'Jahrb. f. wiss. Bot., Bd. xxxi.

contains no nuclei. In the central part of the gonoplasm a dense, deeply stainable substance then appears, sometimes spherical, sometimes irregular in outline, which is regarded by Swingle[1] as a definite organ or organoid of the cell, but which appears to be nothing more than a condensation of granular protoplasm[2]. As soon as it has been produced, one of the nuclei from the periplasm passes into the gonoplasm, and comes into contact with the central body. In *Peronospora parasitica*[3] this nucleus becomes slightly elongated in the direction of the central mass before it reaches it, and sometimes several of the nuclei in the periplasm exhibit a slight elongation in the same direction, so that it is possible that the central mass exerts some attractive power on the periplasmic nuclei, resulting in the passage of one of them bodily through the gonoplasm to come in contact with it.

The antheridium puts out a fertilizing tube, into which one, or perhaps two nuclei pass; this tube then penetrates the periplasm and passes through the gonoplasm until it comes nearly into contact with the female nucleus; and then through an opening at the apex of the fertilizing tube, the male nucleus makes its way into the gonoplasm, and comes into close contact with the female nucleus. The two nuclei may at once fuse to form the zygote-nucleus (*Cystopus candidus*, *C. Portulacae*, *Peronospora Ficariae*, &c.), or the fusion may be delayed for some time, until the thick membrane surrounding the zygote has been partly formed (*Peronospora parasitica*).

The zygote-nucleus, of those species in which the fusion of the sexual nuclei takes place at an early stage, begins at once to divide, and this division is repeated until as many as thirty-two nuclei may be formed; and in this condition it enters on its period of rest. The ripe zygote is therefore multinucleate[4]. In *Peronospora parasitica*, on the other hand,

[1] W. T. Swingle, Two New Organs of the Plant Cell. See Bot. Gazette, Feb. 1898, p. 110.

[2] Wager, loc. cit., 1896.

[3] The following observations on *P. parasitica* are from an unpublished paper by the writer.

[4] Wager, loc. cit., 1896; Berlese, loc. cit.

in which the fusion of sexual nuclei does not occur until a much later stage, subsequent division of the zygote-nucleus does not take place, so that the ripe zygote contains only one nucleus.

This difference in the behaviour of the nucleus during the maturation of the oospore is probably connected with the mode of germination. De Bary[1] has already pointed out that in *Cystopus* and some other species the oospore on germination produces at once a mass of zoospores. In *Peronospora Valerianellae* and others the oospore at once develops a germ-tube. It may be therefore that the uninucleate condition of the zygote indicates germination by a germ-tube, the multinucleate condition germination by the formation of zoospores.

In the Saprolegnieae it has not been found possible to obtain perfectly satisfactory information as to the nature of the sexual process. According to the views of De Bary[2], Marshall Ward[3], Hartog[4] and others, the antheridial tube never opens into the oosphere, although it becomes firmly attached to it. Trow[5], on the other hand, maintains that in some species at any rate a true fertilization occurs by the passage of a nucleus into the oosphere. The facts which he brings forward to support his view are: (1) The young oosphere at first contains only one nucleus. At a later stage two are to be found. (2) These two nuclei fuse together into one, the embryo-nucleus. (3) In one case the apex of an antheridial tube containing a nucleus was observed penetrating the oosphere. (4) The second nucleus of the oosphere appears at an early stage just beneath the wall of the oosphere, and in close proximity to the antheridial tube outside.

From my own observations I am able to confirm (1), (2),

[1] Fungi, Eng. edit., p. 135.

[2] De Bary, Fungi, Eng. edit.

[3] Marshall Ward, loc. cit., p. 272, also Q. J. M. S., 1883.

[4] Hartog, Cytology of the Veg. and Rep. Organs of the Saprolegnieae. Trans. R. Irish Acad., vol. xxx, 1895.

[5] Trow, Ann. of Botany, ix, 1895, and xiii, 1899.

and (4), but I have never seen the penetration of the oosphere by an antheridial tube, although I have observed hundreds of sections of oospheres in all stages of development. At the same time it seems difficult to escape coming to the conclusion that fertilization does take place, and Hartog himself says:—'In a few cases, however, I have seen two nuclei of uneven size in the young oosphere; and from this I might have inferred sexual fertilization, had not the ooangium is these very cases happened to be free from all signs of antheridial branches, let alone "fertilizing tubes[1]." ' Hartog contends that the nuclei of the oogonium fuse together in groups to form the nuclei of the oospheres, and that this is in fact a fusion of potential gametes or sexual cells, which not only takes the place of the morphologically sexual fusion, but is equivalent to it in the sense that it rejuvenates the cell. The presence of two nuclei in the oospheres represents to him the last stage in this fusion of nuclei, and is not a fertilization process as Trow describes. The whole matter evidently requires further investigation before we can come to any definite conclusions as to the exact nature of the phenomena of fertilization in this group.

Among the Chytridineae the only form in which the cytological features of fertilization have been observed is *Polyphagus Euglenae*. Individual plants of this species are unicellular and uninucleate. The formation of the zygote takes place by the fusion of the protoplasm and nuclei from two slightly unequal cells, which are brought into communication with one another by means of a pseudopodium-like process, put out from the smaller (male) cell, which comes into contact with the larger (female) cell. At the point of contact the apex of the pseudopodium swells up to form the zygote cell. The protoplasm and nucleus from the male cell then pass into it, and subsequently the protoplasm and nucleus from the female cell. The two nuclei are at first unequal in size, the male nucleus being the smaller; and it

[1] Hartog, The Alleged Fertilization in the Saprolegnieae. Annals of Botany, vol. xiii, 1899, p. 450.

also contains a smaller quantity of stainable substance. The male nucleus increases in size until it attains the same size and staining properties as the female nucleus, and in this condition the zygote enters on a resting stage. Fusion does not definitely take place until germination begins, and in all cases where it has been seen, it occurs not in the zygote itself, but in the young sporangium which is at once formed when the zygote begins to germinate [1].

In the Mucorineae the zygotes are formed by the fusion of multinucleate cells (apocytia), which are, so far as can be seen, identical with one another. The investigations of Dangeard and Maurice Léger [2] on *Sporodinia grandis* show that the sexual cells are distinctly isogamous and contain several hundreds of minute nuclei. On fusion taking place the protoplasm and nuclei of the two cells become mixed together. Changes then take place leading to the disappearance of all the nuclei. At the same time two groups of deeply stained bodies appear, one at each end of the zygote. According to Léger [3] each group is composed of from fifteen to thirty small spheres (nucleoli?), 'sphères embryogènes.' They arrange themselves into a spherical layer surrounding a globule of oil and then fuse together, producing a hollow sphere, 'sphère embryonnaire,' full of oil. In the process of germination these two 'sphères embryonnaires' increase in size and fuse together. The fused mass becomes clear, and numerous nuclei appear in it, which pass into the sporangiferous mycelium and begin to divide. The same phenomenon is observed in the azygospores, except that only one sphere is produced.

The union of the 'sphères embryogènes' is the sexual process. The azygospores are therefore truly sexual, and

[1] These observations are taken from an unpublished paper by the writer. See also Brit. Ass. Reports, 1898, p. 1064.

[2] Dangeard and Maurice Léger, (1) Recherches sur la Structure des Mucorinées; (2) La Reproduction sexuelle des Mucorinées. Le Botaniste, vol. iv, 1894–5, pp. 4 and 7.

[3] Léger, Structure et développement de la zygospore du *Sporodinia grandis*. Revue générale de Botanique, t. vii, 1895, p. 481.

the process of conjugation is of secondary importance, and not sexually significant in the group [1].

Dangeard [2], however, does not agree with Léger's interpretation of the 'sphères embryonnaires.' For him the ultimate fate of the nuclei has not been determined.

In the Entomophthoreae we also have fusion of similar cells formed on different filaments, but the only genus in which the cytology of the process of conjugation has been investigated is *Basidiobolus* [3], which presents a feature of peculiar interest, in that its zygotes are normally formed by the fusion of adjacent cells (as sometimes occurs exceptionally in the Alga *Spirogyra*), which are uninucleate. Unlike *Spirogyra*, however, the nuclei before fusion undergo mitotic division into two. One daughter nucleus in each cell becomes cut off and degenerates, the other two nuclei with their protoplasm fuse together to form the zygote. The fusion of nuclei takes place at a late stage, and Raciborski has shown that it may even be delayed until germination has taken place; in such cases the germ-tube then contains two nuclei.

Hartog [4] regards the adjacent cells as progametes, 'each of which by unequal divisions forms two gametes, the apical one arrested, the other functional.'

Higher Fungi.

In the Ascomycetes the single nucleus of the young ascus is produced by the fusion of two or more cells derived from the mycelium [5]. Dangeard, who has investigated a large

[1] Léger, loc. cit.

[2] Dangeard, Considérations sur les phénomènes de reproduction chez les Phycomycètes. Le Botaniste, vol. iv, 1894–5, p. 249.

[3] Eidam, Cohn's Beiträge zur Biol. d. Pflanzen, Bd. iv, p. 181. Fairchild, Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum* Eidam, Jahrb. f. wiss. Bot., xxx, 1897, p. 285. Raciborski, Ueber den Einfluss äusserer Bedingungen auf die Wachsthumsweise der *Basidiobolus ranarum*. Flora, 1896, p. 107. Chmielewskij, see Bot. Centralbl., vols. xxxviii and l.

[4] Hartog, loc. cit., Q. J. M. S., p. 27.

[5] Dangeard, La Reproduction sexuelle des Ascomycètes. Le Botaniste, sér. iv, 1894–5. Harper, Beiträge zur Kenntniss der Kerntheilung und Sporenbildung im Ascus, Ber. d. Deut. Bot. Ges., 1895.

variety of forms, states that in the Exoasci the mycelium becomes divided into cells, each of which possesses two nuclei and has the value of an oogonium. The two nuclei fuse together, and the cell becomes an ascus, the single nucleus of which divides to form the nuclei of the spores. In *Peziza*, *Helvella*, *Morchella*, and *Acetabula* the oogonium appears to be formed by apical fusion of two filaments, but this is not the correct interpretation, for Dangeard finds that it is an appearance brought about by the curving of a single filament. But he admits that it may be due to a terminal fusion of two filaments in the case of *Eremascus*[1] and *Dipodascus*[2]. The oogonia always contain two nuclei; two gametes which fuse together to form the nucleus of the oospore.

In *Endocarpon miniatum* the papilla which forms the ascus contains two nuclei, and in *Aspergillus glaucus* the young asci contain two nuclei which apparently fuse together.

Harper[3] shows that in *Peziza Stevensoniana* the ascus-nucleus is formed by a fusion of four nuclei.

Dangeard also states that in the Truffle[4] the cells which form the asci contain two nuclei. These cells appear frequently to arise from the fusion of two different threads, but Dangeard thinks that, as in other cases, it may be simply due to the curving of a single filament. The two nuclei fuse together and at once begin to divide to form the spore nuclei.

This fusion of nuclei in the ascus, or in the cell from which the ascus arises, is therefore apparently general throughout the group, although it is desirable that this should be confirmed, and is regarded by Dangeard as a true sexual process, a fusion of two nuclei of different origin into a single sexual nucleus. He is thus opposed to De Bary's views as to the nature of the sexual process in this group.

[1] Eidam, Zur Kenntniss der Entw. bei den Ascomyceten. Beitr. z. Biol. d. Pflanzen, Cohn, iii, 1883.

[2] Lagerheim, *Dipodascus albidus*, eine neue geschlechtliche Hemiascee, Jahrb. f. wiss. Bot., xxiv, 1892.

[3] Harper, loc. cit.

[4] Dangeard, La Eruffe: Rech. sur son développement, sa structure, sa reproduction sexuelle. Le Botaniste, iv, 1894–5, p. 63.

De Bary's views, however, have been confirmed by the researches carried on by Harper[1] on some of the simpler forms of the Ascomycetes. In *Sphaerotheca*, for example, Harper finds that the organs regarded by De Bary as sexual, oogonium and antheridium, each contain one nucleus, and that the antheridial nucleus passes into the oogonium, the two nuclei then fusing together to form the sexual nucleus of the oospore. The antheridial nucleus is at first slightly smaller than the nucleus of the oogonium, but before fusion takes place it attains practically the same size.

The fertilized oogonium (oospore) becomes surrounded by filaments arising from the basal cells. Then the nucleus divides, and a row of cells forming the ascogonial filament is formed; one of these, the subapical cell, contains two nuclei, and from this cell the single ascus arises. This cell increases in size, and the two nuclei which it contains fuse together into one, and this subsequently divides to form the nuclei around which the ascospores are formed.

Dangeard[2], however, does not accept Harper's results as to the primary sexual fusion of nuclei, although he agrees in general with him as to the subsequent phenomena. In an exhaustive paper he attempts to prove that only one fusion of nuclei takes place, viz. in the ascus. He affirms that the antheridial cell undergoes degeneration at an early stage, which extends both to protoplasm and nucleus; that the ascogonium often contains one nucleus only, even when the covering branches begin to surround it; that ascogonia with two nuclei sometimes show a nucleus still in the antheridial cell, and that an appearance of fusion which he once observed was due not to actual fusion, but to one of the covering hyphae lying alongside the antheridial cell.

From a careful examination of the figures given both by Dangeard and Harper, it appears to me that not only is the

[1] Harper, Die Entwickelung des Peritheciums bei *Sphaerotheca Castagnei* Lev. Ber. d. Deutsch. Bot. Ges., xiii, 1895, p. 475. Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten. Jahrb. f. wiss. Bot., xxix, 1896, p. 655.

[2] Dangeard, La Reproduction sexuelle dans le *Sphaerotheca Castagnei*. Second Mémoire sur la Reproduction sexuelle des Ascomycètes. Le Botaniste, v, 1895–6.

evidence strongly in favour of regarding these organs as sexual, but that it supports Harper's conclusions as to fertilization. Dangeard [1], in fact, himself, in speaking of his own view of the sexuality of *Sphaerotheca*, says :—' Cette fécondation (nuclear fusion in the ascus) laisse même toute liberté d'interprétation au sujet des archicarpes et des branches anthéridiennes ; elle exige simplement que ces organes soient devenus inutiles ; s'ils remplissent encore leur fonction, s'il y a fertilisation, l'asque doit en provenir directement ; et c'est bien le cas, ainsi qu'en témoignent les *Eremascus* Eidam et les *Dipodascus*.'

It is of course possible that fertilization may not always take place in individuals of this species, which would account to some extent for the discrepancy between the observations of these two authors ; and in this connexion the observations made by Mary M. Nichols [2] on certain pyrenomycetous Fungi, which distinctly support the views maintained by Harper, are of considerable interest. The author states that in *Ceratostoma brevirostre* and *Hypocopra* the antheridia fuse with the archicarps in some cases, whilst in others the archicarps appear to develop without fertilization. In *Teichosporella* there remains only a possible rudiment of an antheridium, while in *Teichospora* this has often entirely disappeared.

In the Ustilagineae Dangeard [3] states that a fusion of two nuclei takes place in the young resting spores (brand spores) of *Doassansia Alismatis* and *Entyloma Glaucii*, Dang. *Doassansia Alismatis* forms brown pustules on the leaves of *Alisma Plantago*, and its slender mycelium is found in abundance in the intercellular spaces of the mesophyll. Each cell contains several nuclei. In *Entyloma Glaucii* [4] also the mycelium is found abundantly in the intercellular spaces of the host-plant, and the cells are multinucleate. In both species the

[1] Dangeard, loc. cit., p. 252.

[2] Mary M. Nichols, The Morphology and Development of certain pyrenomycetous Fungi. Bot. Gazette, vol. xxii, 1896, p. 301.

[3] Dangeard, Recherches sur la Reproduction sexuelle des Champignons. Le Botaniste, iii, 1893-4, p. 221.

[4] Dangeard, La Reproduction sexuelle de *l'Entyloma Glaucii* (Dang.). Le Botaniste, iv, 1894–5, p. 12.

resting spores are formed as intercalary swellings on the hyphae, or as terminal swellings on short lateral branches. In the young condition each resting spore contains two nuclei, which fuse together into one. The spores then become surrounded by thick cell-walls. Dangeard has also observed two nuclei in the young spores of *Ustilago*, and he thinks there are two in *Urocystis*. In *Tilletia* he could not obtain stages young enough for the examination of the young spores.

In the Uredineae the researches of Sappin-Trouffy [1] and Poirault and Raciborski [2] give a very full account of the cytology of numerous species of this group in all stages of development. In the aecidia the sporiferous filament contains two nuclei, which both divide at the same time, and by a successive series of parallel divisions a row of spores and intercalary cells are produced, each of which contains two nuclei. Each aecidiospore thus contains two nuclei of different origin. The uredospores and teleutospores also contain two nuclei of different origin. These are regarded by Sappin-Trouffy as the last stages of a series of parallel divisions which started during the formation of the aecidiospores, and which now fuse together in the cell of the teleutospore into a single nucleus. This final fusion is regarded by Sappin-Trouffy as an act of fertilization, and the teleutospore is therefore equivalent to a zygote.

Massee [3] described in 1888 the presence of sexual organs, antheridium and oogonium, in the *Aecidium* found on *Ranunculus Ficaria*, but this has not been confirmed [4].

In the Basidiomycetes a fusion of nuclei has been observed to take place in the basidia of a large number of species

[1] Sappin-Trouffy, La pseudo-fécondation chez les Urédinées et les phénomènes qui s'y rattachent. Le Botaniste, iii, 1893–4. Sur la signification de la Fécondation chez les Urédinées. Le Botaniste, v, 1896–7, p. 32. Recherches Histologiques sur la Famille des Urédinées. Le Botaniste, v, 1896–7, p. 59.

[2] Poirault et Raciborski, Sur les noyaux des Urédinées. Compt. Rend. d. l'Acad. d. Sci., cxxi, p. 308, and Jour. Bot., ix, 1895.

[3] On the Presence of Sexual Organs in *Aecidium*. Ann. Bot., vol. ii, 1888, p. 47.

[4] See Nypels, Bull. d. l. Soc. Belge Microsc., 1885, p. 70.

belonging to the orders Hymenomycetes, Tremellineae, and Auricularieae.

The number of nuclei which fuse together appears to vary in the different species. In *Stropharia stercoraria*[1] two or more ; in *Amanita muscaria*[2] two or three ; in *Mycena galericulata*[3] four, and in *Lepiota mucida*[4] six to eight. Dangeard[5], however, who has extended his researches to species of *Dacryomyces*, *Calocera*, *Craterellus*, *Bovista*, *Nyctalis*, *Hydnum*, and *Polyporus*, states that the young basidium only contains two nuclei. In all these cases the nuclei appear to pass into the basidium from the hymenium, but Rosen comes to the conclusion, from an examination of *Psalliota campestris*, that they may be produced in the basidium.

The details of the nuclear fusion have been observed in *Stropharia stercoraria*[6]. The two nuclei come into contact with one another, and a flattening of each takes place where they touch. A dumb-bell-shaped structure is thus produced. The membrane separating them then disappears, and the nuclear network of the one becomes intermingled with that of the other. The fused nucleus is at first oval in shape, and the two nucleoli for a short time remain separate, but they ultimately fuse together into a slightly elongated mass, which soon becomes spherical, and the nucleus then exhibits no trace of its composite structure. The single basidium-nucleus then divides by mitosis, and the daughter nuclei formed pass through the sterigmata into the spores[7]. The fusion of two nuclei has been observed in *Tremella mesenterica* by Dangeard[8], and in *Auricularia* by Sappin-Trouffy[9]. In both cases the

[1] Wager, Annals of Botany, 1892, p. 146, and 1893, p. 489.

[2] Wager, ibid., 1894, p. 321.

[3] Rosen, Beiträge zur Kenntniss der Pflanzenzellen, ii. Cohn's Beitr. zur Biol. d. Pflanzen, vi, 1892–3, p. 237.

[4] Wager, loc. cit., 1893, p. 321.

[5] Dangeard, Sur la Reproduction sexuelle des Basidiomycètes. Le Botaniste, iv, 1894–5, p. 119.

[6] Wager, loc. cit., p. 496 (1893).

[7] Rosenvinge, Sur les noyaux des Hymènomycètes, Ann. d. Sci. Nat., Bot., sér. vii, tome 3, 1886.

[8] Dangeard, loc. cit., p. 125.

[9] Sappin-Trouffy, Recherches mycologiques. Le Botaniste, v, 1895–6, p. 44.

basidium divides up after the nuclear fusion into a number of cells, each of which contains one nucleus. In *Tremella* the cell divisions are longitudinal; in *Auricularia* they are transverse. Each cell produces a sterigma, at the apex of which a spore is formed, and the nucleus then passes into it.

This phenomenon of nuclear fusion in the Basidiomycetes is regarded by Dangeard as sexual and the basidium as an oospore [1].

Theoretical Considerations.

We have now to consider the general bearing of these facts upon the question of sexuality.

Sexual reproduction may be regarded as a process by which (1) the energy of division is restored, and (2) two independent lines of descent blended into one.

According to many observers the essential end of sexuality is rejuvenescence. The cell in some way becomes enfeebled, loses 'the capacity of carrying on the vital processes by itself [2],' and requires some stimulus to reinvigorate it to further growth. Hence the need for fertilization. But whether this is a primary attribute of living matter or has been secondarily acquired in order to ensure a mixture of germ-plasms derived from different sources has not been determined [3].

A study of the process of fertilization in the Phycomycetes lends support to the view that the fusion of the two cells and nuclei is primarily for purposes of reinvigoration simply. In all the higher forms of life the only mode of reproduction is sexual. In the Fungi and lower forms of life generally we have in addition asexual reproductive organs, which, as Strasburger says [4], 'are especially concerned with the rapid multiplication of the individuals under favourable external conditions; whilst sexual reproduction is of importance in

[1] Dangeard, loc. cit.

[2] Hertwig, The Cell, Eng. edit., p. 291.

[3] See Wilson, The Cell in Inheritance and Development, pp. 129, 130.

[4] Strasburger, The Periodic Reduction of the number of the Chromosomes in the Life-History of Living Organisms. Ann. Bot., viii, 1894, p. 282.

maintaining the existence of the species under circumstances which are unfavourable to the vegetative existence of the individual.'

The number of cases in which there is a blending of two distinct lines of descent into one is rare, and sometimes the conjugating cells are closely related to one another. In *Basidiobolus* the fusion always takes place between adjacent cells. In the Peronosporeae the same filament often gives rise both to the male and female organs, and in some cases this appears to be the rule. In Mucorineae the conjugating cells are borne on separate filaments, but these may be, and often are, branches of the same plant. In *Polyphagus Euglenae* two distinct individuals fuse together, but in *Zygochytrium* the conjugating cells are produced on different filaments proceeding from the same individual. Thus so far as sexual differentiation is concerned, all we can say is that although the conjugating nuclei are in most cases removed at some distance from one another in their development, yet the required stimulus may be obtained when they are as closely related as in *Basidiobolus*.

It has been shown that the need for fertilization in the simpler forms of Fungi and Algae depends to a certain extent upon external conditions, which affect transpiration, atmospheric pressure, and food supply. The connexion between food supply and sexuality in the Fungi was suggested by Marshall Ward [1] in 1884; and Eidam [2] showed that if the conidia of *Basidiobolus* are sown in a nutrient fluid, a firm mycelium is produced, which forms simultaneously both asexual and sexual cells. In an exhausted nutrient medium, on the contrary, the conidia produce only a loose mycelium, which immediately and exclusively gives rise to sexual cells, which unite together to form zygotes.

Again, Raciborski [3], in 1896, working with the same Fungus,

[1] H. Marshall Ward, On the Sexuality of the Fungi. Q. J. M. S., vol. xxiv, 1884. [2] Loc. cit.

[3] Raciborski, Ueber den Einfluss äusserer Bedingungen auf die Wachsthumsweise des *Basidiobolus ranarum*. Flora, 1896, p. 107.

showed that at a low temperature, 6–7° C., only sterile mycelium is formed; that the formation of conidia demands free access of air; and that the formation of zygotes is connected with bad conditions of vegetation, such as the transport of a prosperous culture into an unfavourable medium, dilution of the nutrient medium, or too strong a solution of it, or an elevated temperature.

Klebs[1] showed in his studies on *Sporodinia grandis* that carbohydrates are needed to form zygotes, and that sporangia may be formed luxuriantly in nitrogenous media. Increased transpiration tends to the formation of sporangia. When transpiration is checked within certain limits, zygotes are formed in addition, and when still further checked, zygotes only are formed. When the air-pressure is reduced parthenogenesis results, and if still further reduced no sexual organs are formed at all, and ultimately the production of sporangia also is stopped.

Some observations of my own are also interesting as bearing upon this point. In the case of *Polyphagus*, when there is an abundant food supply in the form of fresh *Euglena* cells, sporangia only are produced. In a very short time, however, as this special food supply becomes exhausted, sexual organs are also formed, and in the later stages of a culture, when the food supply is much reduced, sexual organs only are formed.

In *Peronospora parasitica* and *Cystopus candidus* I find that oospores are mostly developed in those parts from which the food supply has not been absorbed by sporangia, and which are still succulent and full of sap. The portion of the stem which contains them is large and succulent, with few asexual organs present, or they are contained in the young succulent tissues at the apex. Those parts of the plant which are covered with asexual spores and which appear white—those parts where the Fungus is visible to the observer on a cursory examination—very rarely contain sexual organs in abundance.

[1] Klebs, Zur Physiologie der Fortpflanzung einiger Pilze. I. *Sporodinia grandis*. Jahrb. f. wiss. Bot., xxxii, 1898. See also II. *Saprolegnia mixta*. Jahrb. f. wiss. Bot., xxxiii, 1899.

Again, in *Peronospora effusa* the sexual organs are found in abundance in the leaves of the host-plant, but always in greater abundance in the young leaves near the apex. Also in *Peronospora Arenariae*, which is found upon many of the Caryophyllaceae, I soon found that it was useless to look for sexual organs in those parts of the plant which are covered with a luxuriant growth of asexual organs, but that they were only to be found in certain parts of the stem which were slightly differently coloured from the rest, and on which asexual organs were not found in any quantity.

Hartog strongly supports the rejuvenescence theory[1]. He brings forward a considerable body of evidence to show that replacement theories of fertilization are inadmissible, since all fail to account for one or more of the many phenomena involved in the various types of sexual fusion of nuclei. He points out that rejuvenescence is brought about by (1) change of the mode of life, (2) plasmodium-formation, (3) isogamy, involving the fusion of two or more gametes and their nuclei, and (4) oogamy. He further points out that many cases of parthenogenesis involve the fusion of sister nuclei, and that this 'replaces the advent of a male nucleus.' Among the Fungi he instances *Saprolegnia* as a case in which this occurs, but this requires confirmation, as does also the case of *Sporodinia* described by Léger[2], in which he describes the same phenomenon as occurring in the formation of the azygospores.

In animals the polar bodies formed during the maturation of the egg are now generally regarded as reduced ova, or, as Hartog says, they represent true gametes arrested in their development. In certain cases the second polar body remains in the egg, and Boveri discovered 'that in *Ascaris* the second polar body might in exceptional cases remain in the egg, and there give rise to a resting nucleus indistinguishable from the egg-nucleus or sperm-nucleus.' He was thus led to the interesting suggestion that parthenogenesis might be due

[1] Hartog, Some Problems of Reproduction, Q. J. M. S., 1891.

[2] Léger, loc. cit.

to the retention of the second polar body in the egg and its union with the egg-nucleus. The second polar body would thus, in a certain sense, assume the rôle of the spermatozoon, and it might not without reason be said: *Parthenogenesis is the result of fertilization by the second polar body*[1].

This conclusion was confirmed by the observations of Brauer on the parthenogenetic eggs of *Artemia*, in which the second polar body is actually formed, but remains in the egg, and 'here plays the part of a sperm-nucleus precisely as maintained by Boveri[2].'

Here, then, we have a clear case of rejuvenescence taking place by the fusion of two sister nuclei replacing definitely the sexual fusion and producing an egg capable of germination.

Such cases as this are not only very instructive as throwing a light upon the phenomena of sexuality in the lower Fungi, but are very significant when we come to consider the fusion of nuclei which takes place in the reproductive organs of the higher Fungi. That this fusion is not merely a vegetative one, and therefore of little significance, is proved by the fact that not only does it occur generally in all the groups of the higher Fungi, but it takes place at a definite stage in the life-history of the individual, and at a period which immediately precedes the formation of spores. To this there is apparently no exception, and it is therefore evident that we have here a phenomenon of considerable importance in the life-history of the higher Fungi.

Dangeard's view that it is a definite 'sexuality which differs in nothing in its essential characters from that of other plants and animals[3]' is not, I think, justified by the facts, even if we compare it to the sexual act in the higher plants when reduced to its lowest terms, viz. *the fusion of two nuclei* simply. For we find that in some cases a multiple fusion takes place—three, four, and even eight nuclei fusing together to produce the single nucleus of the basidium or ascus. In this respect, therefore, it does not resemble the act of fertilization as we

[1] See Wilson, loc. cit., p. 202. [2] See Wilson, loc. cit., p. 205.
[3] Loc. cit., 1894–5, p. 167.

know it in those cases which have been fully investigated. For there are, I think, no cases in which multiple fusion of nuclei is found as a definite sexual act or accompanying a sexual act, if we except some few cases of polyspermy, in higher plants and animals. And in the lower plants the cases in which it is said to occur are doubtful, if we except those cases of multiple union of motile gametes. In *Sphaeroplea*, for example, it is said that the oosphere-nucleus is formed by the fusion of several nuclei [1]; but Klebahn [2] in a recent paper states that even when the oosphere contains more than one nucleus, they do not fuse together to form one, but that fertilization takes place by the fusion of the spermatozoid-nucleus with one only of the oosphere-nuclei. The presence of more than one nucleus in the oosphere of this plant is remarkable, but, as Klebahn says, it does not alter our views as to the essential phenomena of fertilization, for even here it consists in a fusion of the male nucleus with one nucleus of the oosphere, the other nuclei remaining unchanged [3].

In *Dasycladus* it is said that the nucleus of each gamete is formed by a fusion of several vegetative nuclei [4].

In the Mucorineae, according to Dangeard and Léger [5], a fusion of nuclei apparently takes place in the zygote, but this is not certain, and Istvanffi [6] states that the zygote is 'thatsächlich mehrkernig.' The other cases of multiple nuclear fusion which have been described in the Fungi do not accompany a definite sexual act, although it is suspected to occur in some forms, the cytology of which has not been investigated.

The objection to Dangeard's view which is often made is that the nuclei which fuse are too nearly related to one

[1] See Hartog, Some Problems of Reproduction, p. 19.

[2] Klebahn, Die Befruchtung von *Sphaeroplea annulina* Ag. Sonderabdruck aus der Festschrift für Schwendener.

[3] Klebahn, loc. cit., p. 101.

[4] See Hartog, loc. cit., p. 18.

[5] Loc. cit., Le Botaniste, 1894, p. 10.

[6] Istvanffi, Ueber die Rolle der Zellkerne bei der Entwickelung der Pilze. Ber. d. Deutsch. Bot. Ges., 1895, Bd. xiii, p. 454.

another, coming as they do from one cell, to allow its being regarded as sexual. But, as has already been pointed out, this objection does not hold if we regard the fusion of adjacent cells in *Spirogyra* and *Basidobolus* as sexual. For in these cases the two nuclei may be as closely related as are those of the basidium or the ascus. And the objection would certainly not hold in the case of the Uredineae if Sappin-Trouffy's observations are correct, for the nuclei are far enough removed in relationship, although they are found in the same cell. If with Sachs we could regard each nucleus with its protoplasm as representing a separate unity (Energid), it seems to me that, in the Uredineae at any rate, and possibly in the Ustilagineae and Basidiomycetes, we might consider these nuclear fusions as indicating a sexuality nearly equivalent to that in *Basidiobolus*.

Or we might regard these nuclei with Hartog[1] as the centres of potential gametes, in which case the nuclear fusions would be of the nature of those parthenogenetic fusions which take place in the eggs of *Artemia*, in *Saprolegnia* (if Hartog's observations are correct), in the azygospores of *Sporodinia* (according to Léger[2]), and in *Derbesia*, where the zoospores have nuclei constituted by the fusion of several vegetative nuclei, a process which, according to Hartog[3], replaces the formation and union of gametes. But it seems to me that the evidence before us is not sufficient to enable us to come to any definite conclusion one way or the other, and a most serious objection to Dangeard's view that it is a true sexual process is found in Harper's observations on *Sphaerotheca* and other simple forms of the Ascomycetes. These observations show that we have in these cases two distinct nuclear fusions. The first appears to be morphologically a true sexual fusion of two nuclei from different cells, resulting in the production of an ascogonium; the second, a fusion of two or more nuclei in the ascus which occurs at a definite stage in the life-history, and results in the formation of ascospores. What is the meaning of this second nuclear fusion?

[1] Hartog, loc. cit. [2] Léger, loc. cit. [3] Hartog, loc. cit.

One explanation which seems to me possible is that it is brought about by the subsequent development of the oospore into an ascogonial filament of cells instead of being simply transformed into an ascus, as in *Eremascus*, and that the energy imported by the sexual fusion having become used up in the production of the ascogonial cells, the necessary energy to produce another reproductive cell, the ascus, can only be obtained by a further nuclear fusion.

In the higher Ascomycetes this second nuclear fusion has probably replaced altogether the morphologically sexual fusion of the simpler forms, and cannot therefore be regarded as a true sexual phenomenon, though perhaps, as Groom [1] points out, presenting some analogy to the sexual process, but not homologous with it.

The only phenomenon which bears directly upon this double fusion of nuclei in the Ascomycetes is that described by Chmielewskij in *Spirogyra crassa* [2]. In this Alga the two sexual nuclei fuse together in the zygote; the resulting nucleus then divides, by karyokinetic division, into four, of which two break up into fragments and disappear, while the other two, the secondary nuclei, again unite into the definite nucleus of the zygote, which remains till germination.

If the phenomenon, as described by Chmielewskij, really takes place, it resembles in a striking manner what occurs in *Sphaerotheca*. For in both cases we have first a fusion of sexual nuclei, then division into four or more, and subsequent fusion of two of this again into a single nucleus. Looked upon in this light, the second nuclear fusion would then be a sexual phenomenon in *Sphaerotheca*, equivalent to the second fusion in the zygote of *Spirogyra*. And it is conceivable, I think, that an equivalent of this second fusion may have replaced altogether the primary fusion, so that in the higher Ascomycetes the second is the only one which

[1] Groom, On the Fusion of Nuclei among Plants: A Hypothesis. Trans. and Proceedings of the Botanical Society of Edinburgh, 1898, p. 132.

[2] Chmielewskij, Materialien zur Morphologie und Physiologie des Sexualprocesses bei den niederen Pflanzen. Charkow, 1890 (Russisch), Referat in Bot. Centralbl., l, 1892, p. 264.

occurs; that is to say, the ascogonial nucleus, although unfertilized, may still possess sufficient energy to produce the ascogonial filament, but the necessary stimulus for the production of ascospores must be brought about by the fusion of two nuclei in those ascogonial cells from which asci arise.

We might also regard the fusion in the Basidiomycetes as having arisen in the same way, although in this group we have no indication of the primary sexual process.

Groom [1] concludes that the nuclear fusions in question are asexual in character, and superposed upon and subsequent to the sexual act. They are concerned with the alternation of generations, and take place in a small fructificative degenerate generation.

In the Uredineae and Ustilagineae we have no indication whatever of a primary sexual process; but from the fact that these groups have become so completely parasitic—a parasitism of a peculiarly high order, as Marshall Ward [2] puts it, by which the organism has adapted its life to the habits of its host—we should expect also some modification in the sexual organs, so that here, as in the Basidiomycetes and Ascomycetes, we are probably dealing with a degenerative nuclear fusion, not homologous with the sexuality of the Phycomycetes.

It is not possible to come to any final conclusion as to the exact significance of these nuclear fusions in the higher Fungi. That they take the place of a sexual act, and lead to the reinvigoration of the cell, cannot, I think, be doubted.

As Strasburger says: 'If the nuclei which fuse proceed from parts of the plant far removed from one another, one could see in this fusion a re-establishment of equilibrium necessary for the preservation of the species, which would be comparable in its physiological effects to fertilization [3].'

But even if the nuclei are found not to 'proceed from parts

[1] Groom, loc. cit. [2] Loc. cit., p. 296.

[3] Strasburger, Ueber per. Red. der Chromosomenzahl im Entwicklungsgang der Organismen. Biol. Centralbl., xiv, 1894, p. 864. See also Hartog, loc. cit., Q. J. M. S., p. 69.

of the plant far removed from one another,' it seems to me that we could still regard it as comparable physiologically to fertilization, just as in *Artemia* the fusion of the second polar body with the egg-nucleus may be regarded as physiologically a process of fertilization. In the present state of our knowledge, however, we cannot, I think, regard it with Dangeard as a morphologically sexual phenomenon, especially in the light of Harper's researches.

But it explains, I think, in a satisfactory manner, what has always been somewhat of a mystery—how it is that the asexual reproductive cells of the Fungi become stimulated to further growth and development; and Strasburger's statement that 'these arrangements for asexual reproduction were so efficient in the Fungi that the result was the disappearance of the sexual organs and of sexual reproduction [1],' receives a further interpretation.

Conclusions.

The following summary of facts and conclusions drawn therefrom indicates the scope of this paper.

1. In the Phycomycetes we have a true sexuality, consisting in the fusion of two nuclei derived from separate more or less completely differentiated cells. In its essential characters it does not appear to differ from that of higher plants and animals.

2. Before fusion takes place there may be a preliminary division of the sexual nuclei (Peronosporeae, *Basidiobolus*), or it may be absent (*Polyphagus*), but in this case the nuclei before fusion lose a considerable amount of stainable substance, which appears to pass into the surrounding cytoplasm.

3. This preliminary division may be connected with chromosome reduction, but the evidence before us is not sufficient to enable us to come to any definite conclusions as to its theoretical significance. Moreover, the reduction in the number

[1] Strasburger, The Periodic Reduction of the number of the Chromosomes, &c., Ann. Bot, vol. viii, 1894, p. 283.

of the chromosomes is stated to take place on the germination of the zygote in the Peronosporeae (Berlese).

4. Before fusion the two nuclei, which at first may differ very much in size (*Polyphagus*), attain the same size and staining properties.

5. Fusion may take place at once, or may be delayed until after germination has taken place (*Basidiobolus*, *Polyphagus*), so that apparently the formation of the oospore membranes and the early stage of germination are independent of nuclear fusion.

6. The fusion of nuclei takes place in the resting condition (*Cystopus candidus*, *P. parasitica*, *Basidiobolus*), or possibly, in some cases, in the chromosome stage (species of *Cystopus* and *Peronospora* (Berlese), *Polyphagus* (Wager)).

7. The formation of sexual organs depends to some extent upon the conditions under which the Fungus is grown.

8. Centrosomes have not been observed taking part in the process of fertilization. Definite centrosomes have not been observed in the Phycomycetes.

9. The sexual elements which fuse together in any given case are generally derived from one and the same individual, often from the same filament; and in *Basidiobolus* the two nuclei which fuse are derived from adjacent uninucleate cells.

10. In the higher Fungi nuclear fusions occur at a definite stage in the life cycle, resulting in the production of spores either directly (Ustilagineae and Uredineae) or indirectly (Ascomycetes and Basidiomycetes).

11. These nuclear fusions are probably not morphologically sexual, but they replace the sexual act, and are physiologically equivalent to it, in that the cell is thereby reinvigorated to further development, and this accounts for the continued asexual reproduction of these forms.

12. Among the higher Fungi the simpler Ascomycetes only, such as *Sphaerotheca*, exhibit a true sexual fusion, accompanied, however, by a subsequent fusion of nuclei in the ascus.