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MSS. intended for publication and books, etc., intended for review should be sent to Professor J. McKeen Cattell, Garrison-on-Hudson, N. Y.

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THE EVOLUTION OF A BOTANICAL PROBLEM

THE HISTORY OF THE DISCOVERY OF SEXUALITY IN PLANTS[1]

FROM the beginning of man's thoughtful consideration of natural processes, the phenomenon of sexual reproduction, with the associated phenomena of heredity, have persistently engaged his keenest interest. The primary fact of the necessary concurrence of two individuals in the production of offspring was, in the case of animals, recognized from the beginning. The equivalent phenomenon was not established for plants until the end of the seventeenth century. At this time, however, little more was known of the essential features of the sexual process in animals than had been familiar to Assyrians, Egyptians and Greeks twenty centuries before.

Of the additions made since 1700 to our knowledge of sexual reproduction, of its varied types and of the associated phenomena, no mean share has been contributed by botanical investigators. Noteworthy among such contributions are the work of Koelreuter and Mendel in the production and systematic study of plant hybrids, and the early work of Pfeffer on the chemotactic response of spermatozoids. Of more recent work we may cite that of the plant cytologists on apogamy and apospory, on multi-nucleate sexual cells or gametes, and on the long-delayed nuclear fusion in the sexual reproduction

[1] Address of the vice-president and chairman of Section G, Botany, American Association for the Advancement of Science, December, 1913.

of the plant rusts. It should then be of interest for us to consider just how and when the more important steps have been taken in building up the vast mass of somewhat incomplete knowledge that we now possess concerning the reproductive process in plants. Because of exigencies of time and patience, I shall confine myself primarily to an attempt to picture the chief steps by which our present knowledge of the essential sexual process, the union of two parental substances, has been attained. Incidentally we may note the changes in point of view of investigators and in their mode of attack on this problem. I shall attempt to suggest the trend of development more clearly by often grouping the chief phenomena discovered in such a way as to indicate the sequence of discovery, within each group, of the different phases of the sexual process, though the order of discussion may thus not always accord with the sequence, in time, of the discovery of individual phenomena in plants as a whole.

In following the evolution and change in aspect of our problem we shall often find it best to keep a few relatively great names prominent. This will serve in the first place to make the story more vivid and intelligible. It will at the same time often come nearer the essential truth, for in each great forward step some one worker has usually been the dominating leader.

I. THE DISCOVERY THAT POLLINATION IS A PREREQUISITE TO SEED-FORMATION. 750 B.C. TO A.D. 1849

The first discoveries pointing to the existence of sex in plants were evidently made very early in human history by peoples cultivating unisexual plants for food. The existence of fertile and sterile trees of the date palm, *e. g.*, was known to the peoples of Egypt and Mesopotamia from the earliest times. Records of the cultivation of these trees and of artificial pollination have come down to us on bas-reliefs from before 700 B.C. found in the palace of Sargon at Khorsabad (Haupt and Toy, 1899). The Assyrians, it is said, commonly referred to the two date trees as male and female (Rawlinson, 1866). The Greeks, in spite of their peculiarly keen interest in natural phenomena, failed to offer any definite interpretation of this well-known fact concerning the date palm. Aristotle and Theophrastus report the fact, gained apparently from the agriculturalists and herb-gatherers, that some trees of the date, fig and terebinth bear no fruit themselves, but in some way aid the fertile tree in perfecting its fruit. But without recording a single crucial experiment on the matter, Theophrastus concludes that this can not be a real sexuality, since this phenomenon is found in so few plants.

In this uncertain state the knowledge of sexuality in plants was destined to rest for twenty centuries, waiting for the experimental genius of Camerarius to give a conclusive answer to the question raised by the Assyrian and Greek gardeners and answered wrongly by Theophrastus. The English physician Grew (1676) did, it is true, accept and expand the suggestion of Sir Thomas Millington that the stamens serve as the male organs of the plant. Thus Grew concludes (p. 173) that when the anther opens the "globulets in the thecæ act as vegetable sperm which falls upon the seed-case or womb and touches it with prolific virtue." But this guess, though it proved correct in the main point, was still a guess, and not supported by any critical evidence so far as recorded by Grew. The only adequate evidence that could be obtained on this question, while microscopes and technique were still so imperfect, was experimental evidence. This kind of proof was first given, some 20 years after Grew's work, by Rudolph Jakob Camerarius, of Tübingen. Camerarius fully appreciated the presence of a real problem here. He also had the genius to see that the philosophical attempts of many of his immediate predecessors, to discover its solution entirely in their own inner consciousnesses, were futile. With the insight of a modern experimenter Camerarius put the question to the plants themselves. The results of his experiments, as reported in the famous letter of 1694 to Professor Valentin, of Giessen, were clear and conclusive. After noting that aborted seeds were produced by isolated—and therefore unpollinated—female plants of *Mercurialis,* and of the mulberry; by castrated plants of the castor bean; and by plants of Indian corn from which he had removed the stigmas, Camerarius gives his interpretation of these phenomena. He says (Ostwald "Klassiker," p. 25):

In the vegetable kingdom there is accomplished no reproduction by seeds, that most perfect gift of nature, and the usual means of perpetuating the species, unless the previously appearing apices of the flower have already prepared the plant therefor. It appears reasonable to attribute to these anthers a noble name and the office of male sexual organs.

In the seventy years after Camerarius had proved in this way the existence of two sexes, and the fertilizing function of the pollen in plants, little advance was made. Bradley, of London, Gleditsch, of Berlin, and Governor Logan, of Pennsylvania, confirmed parts of Camerarius's work, and the great Linnæus accepted the conception of the stamens and pistils as sexual organs as clearly proven, *not,* be it noted, *by the results of Camerarius's experiments* but by "*the nature of plants.*"

In 1761, J. G. Koelreuter, of Carlsruhe, published an account of the first systematic attempt that had been made, with either plants or animals, to produce and carefully study artificial hybrids. In his work with hybrid tobaccos, he demonstrated that characters from both parents are often associated in a single offspring. He thus not only completed Camerarius's work, but also, by showing that the male parent participates in the makeup of the offspring, he helped materially to break down the "emboitement theory" of Christian Wolff, which assumed that the embryo came entirely from the egg, and that its characters could not be influenced by the male parent. It is true that Koelreuter was mistaken in believing that fertilization is accomplished by the mingling of the oil on the pollen grains with the secretion of the stigma to form a mixed fluid, which he supposed then penetrated to the ovule. Nevertheless, his conception of the mingling of two substances was a move with the proper trend.

Koelreuter also demonstrated that in nature the pollen necessary to fertilization is often brought to the stigma by insects. He thus opened up a field of research which was cultivated with such splendid effect by Konrad Sprengel thirty years later, and by Darwin, Müller and others a century afterward.

In spite of the absolutely conclusive work of Camerarius, Koelreuter and Sprengel on the sexuality of plants, their conclusions were often rejected during the first half of the nineteenth century. Certain devotees of the nature philosophy, for example, occupied themselves either in proving over again, after Cesalpino, that plants can not be sexual, *because of their nature,* or in trying, by ill-conceived, and carelessly performed "experiments," to prove the conclusions of Camerarius and Koelreuter erroneous. These objectors were finally silenced, however, when Gaertner, in 1849, published the results of such a large number of well-checked experiments, entirely confirming the works of Camerarius, Koelreuter and Sprengel, that no thinking botanist has since doubted the occurrence in flowering plants of a sexuality essentially identical with that found in animals.

II. THE DISCOVERY OF THE POLLEN TUBE AND ITS RELATION TO THE ORIGIN OF THE EMBRYO, 1823–1847

During the opening years of the nineteenth century a number of botanists, who believed in the sexuality of plants, tried to discover by the aid of the microscope just how fertilization is effected. Most botanists of the day believed the pollen grain burst on the stigma, and that its granular contents found a way through the style to the ovary. An entirely new aspect of the problem of fertilization was opened up, however, when in 1823 Amici, of Modena, saw on the stigma of *Portulacca,* young pollen tubes arising from the pollen grains. Seven years later he followed these tubes through the style to the micropyle of the ovule. At about this time also, Jakob Matthias Schleiden (1838) took up the study of this same problem. He was a man of vigorous intellect and great versatility, who sometimes misinterpreted what he saw, but who proved a most stimulating opponent to a number of other workers who did observe accurately. After denying Robert Brown's assertion that the pollen tubes of the orchids arise in the ovary, Schleiden proceeded to describe and figure the pollen tube as penetrating, not merely the style and then the micropyle, but even far into the embryo sac itself.

Here, as he says in his Grundzüge (II., p. 373):

> The end (of the pollen tube) soon swells, either in such a way that the vesicle arising in it fills the whole cavity of the portion of the tube within the embryo sac, or there is left, between the apex of the embryo sac and the embryonal vesicle of the tube, a long or a short cylindrical portion of the latter, the suspensor.

He thus regarded the embryo sac as a sort of hatching place for the embryo which he thought formed from the end of the pollen tube. This idea of the origin of the embryo really denied the occurrence of any actual sexual process, and made the pollen the mother of the embryo.

In 1846, however, the error of this conception was clearly demonstrated by Amici, who showed that the embryo of the orchids arises from an egg which is already present in the embryo sac when the pollen tube reaches it. It is this pre-existing egg, according to Amici, that is stimulated to form the embryo by the presence near it of the pollen tube. This view was confidently supported by Mohl (1847) and Hofmeister (1847) in the following year, and the controversy with Schleiden became even more spirited. As Mohl afterward wrote (1863), men were "led astray by their previous conceptions to believe they saw they could not have seen." The dispute even approached the acrimonious, as when Schleiden (1843) says of one worker's figures, "Solche Praparate sind ohne Zweifel aus den Kopf gezeichnet."

Hofmeister, from the beginning of his study of fertilization in seed plants, had sought in the pollen tube for some equivalent of the spermatozoids, those motile male cells of the mosses and ferns that had first been understood by Unger in 1837. He was unable, however, to do more than point out the mistake of earlier observers in regarding the starch grains of the pollen tube as spermatozoids, and to suggest the likelihood that these motile cells might be discovered in the gymnosperms, a prediction the fulfilment of which was realized by Ikeno and Webber fifty years later. In his study of pollen tubes Hofmeister demonstrated to his own satisfaction that the tube does not open in accomplishing fertilization. His view, which was the one current till 1884, was that the egg is stimulated to develop into the embryo by some substance that diffuses through the *imperforate* wall of the pollen tube.

III. THE DISCOVERY OF A PROTOPLASMIC FUSION AT FERTILIZATION

We come now to consider a series of discoveries of supreme importance in the investigation of the essential sexual process in plants. This is the period in which the problem that had baffled naturalists for twenty centuries was at last solved, at least in one most essential feature, by the demonstration of the occurrence at fertilization of a mingling of paternal and maternal substances.

It will not be without interest at this point to note the intellectual stimuli which led an unusual number of workers to investigate this phase of our problem.

In the first place there were on record, and under discussion at the middle of last century the many puzzling observations of the "Spiral Faden," or animalculæ, as they were thought to be, that had been found arising from a number of plants. These motile, spiral filaments had been seen in a liverwort (*Fossombromia*) by Schmiedel (1747), in *Sphagnum* by Esenbeck (1822), in *Chara* by Bischoff (1828), and finally, on the fern prothallus by Naegeli (1844). Unger (1834–37) studied these bodies in the mosses (*Sphagnum* and *Marchantia*) and declared his belief that they are not infusoria, but are the male fertilizing cells. At this time also the zoologists of the day were making the first detailed studies of the spermatozoa of animals. Barry (1844) had seen a spermatozoon within the egg of the rabbit; Leuckart (1849) saw them enter the frogs' egg and then, in 1851, Bischoff and Allen Thompson proved that fertilization is accomplished by the actual entrance of the spermatozoon into the egg. A no less important influence, in stimulating the botanical workers on the problem of fertilization, was the magnificent work of Hofmeister, on the reproductive structures of the mosses, ferns and conifers. By these splendid researches he had indicated to men of less insight, and less comprehensive imagination, just the points in the life cycles of plants where the critical phases of the reproductive process are to be sought.

Among the many workers engaged on this problem of fertilization in plants in the third quarter of last century there was, in consequence of readier exchange of information, an attitude of greater consideration for the work of other investigators than was found in the two preceding decades. There were differences of opinion and interpretation, to be sure, but there was less of that strenuous cocksureness when men saw, or thought they saw, differently from others. The mistakes of the brilliant Schleiden were perhaps remembered. Men like Hofmeister, Pringsheim and Strasburger added to and modified the interpretations of other workers in the same spirit with which they remolded their own immature conclusions. There was a spirit of cooperation evident; it became possible for a worker to observe and record the fate of a pollen tube in good temper and with calm judgment.

The first steps toward the demonstration of a union of two masses of living substance at fertilization resulted from the study of a group of plants, the algæ, in which sexuality had not been proven or generally admitted. It had, however, long before, been suggested in the case of *Spirogyra* by Hedwig (1798) and Vaucher (1803).

The algæ were in fact especially advantageous for the study of fertilization, since the development and behavior of the reproductive organs and cells could, without elaborate preparation, be readily seen under the microscope, and often followed through in living material. Thus, Thuret in 1853 for the first time saw the active sperms attached to the egg of *Fucus,* and in 1854 proved experimentally that only eggs to which spermatozoids have had access will germinate. He thus demonstrated in this alga the correctness of Unger's unsubstantiated surmise (1837) that the spermatozoids are the male fertilizing cells. In *Œdogonium,* Pringsheim, in 1856 (p. 9), watched the spermatozoid push into the receptive tip of the living egg and saw the characteristic oospore wall formed in consequence. This, except for the less satisfactory observations made on *Vaucheria* by the same worker a year previous, is the first case recorded of the observation of the actual union of male and female cells in any plant. Such a union of the protoplasmic masses of the two sexual cells was soon shown to be a characteristic feature of fertilization in a number of algæ. Thus de Bary saw it in *Spirogyra* (1858), and Pringsheim (1869) repeatedly observed the gradual fusion of the motile gametes of *Pandorina.* It was nearly thirty years later, however, that this phase of fertilization was first seen in seed-plants by Goroschankin and Strasburger.

The workers on this problem were on the lookout for further details of the process of fusion, and even knew rather definitely what they were looking for, but failed to discover it from lack of proper methods of preparation of material. Thus, *e. g.,* Strasburger, in 1877, carefully studied the process of conjugation in *Spirogyra* and found that "Hautschicht fuses with Hautschicht, Kernplasma with Kernplasma"—"The chlorophyll bands unite by their ends"—and then goes on to say of the feature that evidently interested him most, "the cell nuclei of both cells, however, become dissolved; the copulation product is without a nucleus." Two years later, Schmitz (1879), when studying hematoxylin-stained material of this alga, was more fortunate. He saw the two nuclei in the zygote, as he says, "approach nearer and nearer, come into contact and finally fuse to a single nucleus." This observation by Schmitz is an important one, for in it we have the first clear statement that the nucleus of the male cell passes over intact to the female cell, there to fuse with the female nucleus.

Strasburger had, it is true, seen a second nucleus fusing with that of the egg in the archegonia of *Picea* and *Pinus* in 1877. He did not, however, really know the source of this second nucleus, though he suspected some relation to those that are present earlier in the tip of the pollen tube. These tube nuclei he says are dissolved just before fertilization, and then just after fertilization, to quote (1877):

> The male nucleus *formed from the contents of the pollen tube* is found now near the end of the tube, now near, or in contact with, the egg nucleus. . . . The protoplasmic contents of the pollen tube, I hold, passes through the (imperforate) tube-membrane in a diosmotic manner.

The fertilization of the gymnosperms, because of their large eggs, pollen tubes and nuclei, was then being studied by a number of workers. One of these, Goroschankin, in 1883, was able to demonstrate that in *Pinus pumilio* the pollen tube opens at the end, and that through this pore the two male cells pass bodily into the egg. Goroschankin's mistake, in supposing both male nuclei to fuse with the egg nucleus, was corrected by Strasburger the following year. The latter (1884) saw the same bodily exit of both male nuclei from the *open* pollen tube of *Picea,* but found only one male nucleus fusing with that of the egg. In the same publication Strasburger also records numerous instances in which he had been able to observe the same mode of escape of the contents of the pollen tube into the ripe embryo sac in angiosperms. At last, as Strasburger puts it, in discussing fertilization in the conifers:

> The most important morphological facts are clear. It is established that the male nucleus that copulates with the egg nucleus, passes *as such* out of the pollen tube into the egg.

Thus, finally, was the actual material contribution of both parents to the embryo of the seed plants first seen. This was just two centuries, lacking a decade, after Camerarius (1694) had proven that the presence of pollen on the stigma is indispensable to seed formation. One chief reason why this important problem so long baffled all investigators was the lack of proper methods of preparing material for study. The older method of studying unfixed and unstained sections had certain advantages, it is true. The sequence of developmental stages was often determined with certainty by actually following their succession in living material under the microscope, and there was less cause also for dispute about artifacts. But structures of the same refractive qualities were not readily distinguished in such sections. As Strasburger himself says (1884, p. 18):

> The negative results of my earlier studies and of those of Elfving were due to the lack of a method which permitted the nuclei to be distinguished in the strongly refractive contents of the pollen tube up to the moment of fertilization.

That these studies of 1884 were successful was largely due to the use of material fixed in five-tenths-per-cent. acetic acid, one-per-cent. osmic acid or absolute alcohol, and stained in borax carmine, hematoxylin or iodine green.

The extreme significance of the fact that those most highly organized portions of the cell substance—the nuclei—were so prominent in the process of fertilization was at once appreciated by Strasburger, who in 1884 (p. 77) announced the following general conclusions as the outcome of his consideration of the phenomena observed:

> (1) The fertilization process depends upon the copulation with the egg nucleus of the male nucleus that is brought into the egg, which is in accord with the view clearly expressed by O. Hertwig. (2) The cytoplasm is not concerned in the process of fertilization. (3) The sperm nucleus like the egg nucleus is a true cell nucleus.

In the years since 1884 the nuclei have been found to be the structures chiefly concerned in fertilization whenever such a process occurs. Among the earlier observations of this nuclear union at fertilization in each of the great groups are the following, named in the order of discovery: It was seen in *Pilularia* (Campbell, 1888), in *Riella* (Kruch, 1891), in *Œdogonium* (Klebahn, 1892), in the plant rusts (Dangeard and Spain-Trouffy, 1893), in the toad-stools (Wager, 1893), in the red alga *Nemalion* (Wille, 1894), in *Sphærotheca* (Harper, 1895), in the rockweed, *Fucus* (Farmer and Williams, 1896). Finally Zederbauer (1904) reported it for the Peridineæ and Olive (1907) and Kraenzlin (1907) made it out in the myxomycetes.

The observations just referred to, and many others on plants in all groups, warrant the general application of Strasburger's conclusion that a nuclear union is the characteristic feature of every sexual process. The few cases where the male cytoplasm seems more prominent than usual, as in the three conifers studied by Coker (1903), Coulter and Land (1905) and Nichols (1910), can not yet be said to have rendered it very probable that this cytoplasm plays a primary part as an inheritance carrier.

IV. THE DISCOVERY OF THE ALTERNATION OF GENERATIONS IN PLANTS, 1851–

The fact that the sexual cells of the higher plants are produced on a plant body or individual distinct from that which forms the asexual reproductive cells; and that in the normal life cycle the one type of individual arises from, and later gives rise to, an individual of the other type, must be regarded as one of the most significant features of the evolution of plants yet discovered. One of the chief general results of the magnificent work of Hofmeister was the discovery of this regular alternation of a sexual and an asexual generation, not only in the life history of the mosses and ferns, but also in that of the seed plants. Hofmeister states this result clearly in the *Vergleichende Untersuchungen* and makes it apply still more broadly in a brilliant generalization published in the "Higher Cryptogamia." There he says (p. 439):

> The phenogams, therefore, form the upper terminal link of a series, the members of which are the Coniferæ and Cycadeæ, the vascular cryptogams, the Muscineæ and the Characeæ. These members exhibit a continually more extensive and more independent vegetative existence in proportion to the gradually descending rank of the generation preceding impregnation, which generation is developed from reproductive cells cast off from the organism itself.

Since Hofmeister's day detailed investigations by many workers have fully confirmed Hofmeister's conclusion. They have shown the essential homology, not only of the spore-producing organs, and the one or two kinds of spores produced in them, but also of the structures arising from these spores, throughout all cormophytes, from the mosses upward.

In the studies of the algæ that followed immediately after Hofmeister's work, investigators of these plants sought in them for some evidence of that regular alternation of sexual and asexual phases that had been demonstrated in higher plants. Pringsheim (1856, p. 14) one of the ablest of these students of the algæ, at first regarded the multicellular body, formed at the germination of the oospore of *Œdogonium* and *Coleochæte,* as an asexual phase, comparable with the simple sporophyte of the liverwort *Riccia.* Celakowsky (1886) distinguishes as *homologous alternation* those cases, in algæ, like *Ulothrix* or *Œdogonium,* where the gamete-producing generation seemed capable of zoospore production also. The constant and regular alternation of the archegoniates and seed plants he called *antithetic alternation.* Pringsheim (1877) found that moss protonemata form from cuttings of the seta of the sporophyte as well as from bits of the gametophyte. From this fact, and from Farlow's discovery (1874) that a sporophyte of the fern, *Pteris cretica,* may arise directly from the prothallus, without the fertilization or even the formation of an egg, Pringsheim concluded that both generations of the archegoniates are really identical. He says (1877, p. 6):

> I believe the moss sporogonium stands to the moss plant in the same relation that the sporangium-bearing *Saprolegnias* do to the oogonium-bearing plants of this species, . . . I therefore turn against this interpretation of the fruit generation of the thallophytes in general, and especially against this interpretation of the sexual shoot generation of the Florideæ and Ascomycetes. . . . The cystocarp is evidently not a *separate* individual but part of the sexual plant that produces it.

The antithetic view was reasserted, however, especially by Celakowsky (1877) and Bower (1890), both of whom emphasized the suggestion of A. Braun (1875) that the sporophyte is a new thing phylogenetically. Bower holds that the types of sporophyte found in the archegoniates have arisen by the amplification of the zygote, with the sterilization for vegetative functions of smaller or larger portions of the originally all-pervading sporogenous tissue. The amphibious type of alternation of the mosses and ferns has arisen, according to Bower's conception, with the migration of these plants to the land, and the assumption of the terrestrial habit by the sporophyte. The antithetic view was also supported in a most striking way, later, by the results of the workers on chromosomes.

The homologous view of alternation also has not been without supporters in the years since Pringsheim. One of its upholders, Klebs (1896), based his belief on the fact that he could determine the type of reproductive cells formed by the algæ *Hydrodictyon* and *Vaucheria,* by changing the conditions under which they are grown. Lang (1896–98) favored the homologous view because of the discoveries of Farlow, de Barry, Bower, Farmer and himself on apogamy and apospory. Scott, one of the strongest advocates of the homologous alternation theory, bases his belief not only on the evidence afforded by the cases of apogamy and apospory, but also on the fossil record. He points out the lack of any sporophyte, living or fossil, that can be regarded as ancestral to that of the ferns. In arguing for the homologous origin of the leafy fern sporophyte from a liverwort-like thallus Scott says (1911):

> We know plenty of intermediate stages between a thallus and a leafy stem; but no one ever saw an intermediate stage between a sporogonium and a leafy stem.

V. THE DISCOVERY OF CHROMOSOME REDUCTION AND OF SYNAPSIS, 1888–

We have seen that during the two decades at the middle of last century students of sexuality in plants devoted their attention to the discovery of the relation of the pollen tube to the origin of the embryo. The three decades after 1860 were given largely to the proof of a union of a paternal with a maternal nucleus as a constant feature of the sexual process in plants. For the past two decades workers interested in reproduction have been engaged especially in determining the behavior and fate, in the various phases of plant development, of those essential elements of the nuclei, the chromosomes. The result of this study has been to give us a much more definite criterion than we had before, of just what constitutes a sexual process. Moreover, this intimate examination of the chromosomes, together with the precise means of germinal analysis by breeding, introduced by Mendel, has given us some insight into the significance of the sexual process in the ontogeny and phylogeny of plants.

The discovery of chromosomes in plants may best be attributed to Strasburger, who, in 1875, first figured them distinctly in the embryo of *Picea.* It is true that Hofmeister (1867) had noticed the equatorial plate of "albuminous clumps" in cells at the time of their division, and Russow (1872) saw, in the dividing spore mother-cell nuclei of *Ophioglossum,* plates of vermiform rods ("Stäbchenplatten"). Strasburger (1879) and Hanstein (1880) and Flemming (1880) were, however, the first to realize the constancy of the occurrence of chromosomes in the dividing plant nucleus. The fact soon pressed itself upon investigators that the number of these chromosomes differs in different plants, and in different phases of the same plant. Then followed the epoch-making discovery of the zoologist Van Beneden (1883), that the number of chromosomes in the egg and sperm of *Ascaris* is the same, and that the double number characteristic of the body cells becomes reduced during the maturing of the germ cells. Botanists after some delay, due, as Strasburger says, to lack of proper technique, succeeded in demonstrating these same facts for plants. Thus Strasburger in 1888 showed that the number of chromosomes characteristic of the egg and of the male nucleus in a number of angiosperms is the same, and is fixed by a reduction, occurring in the mother cells of the pollen and of the embryo-sac. Guignard also (1889 and '91) demonstrated these phenomena in *Lilium* and in the pollen mother cells of *Ceratozamia,* noting the eight double chromosomes in the latter and other peculiarities of the first mitosis. Overton (1893) counted the same number of chromosomes in the female prothallus of *Ceratozamia;* while Farmer (1894) found four chromosomes in the thallus and sexual reproductive cells of *Pallavicinia* and eight in the seta and capsule.

Later in the same year Strasburger, in a masterly address before the British Association, completed the proof of Overton's suggestion (1893) that reduction in the mosses and ferns also takes place, as Overton puts it, "in the mother-cells of the spores; that is, at the point of alternation of the generations." Strasburger, by comparing his counts of chromosomes in the dividing spore mother cells of *Osmunda,* with the number seen by Humphrey (figures published in 1895) in the tapetal cells, found the latter number about double. It is interesting to note also that the *Osmunda* slides used in this work were among the first paraffin sections used by Strasburger.

From this correspondence of the liverwort and fern mentioned with the seed plants in which reduction had been seen, Strasburger went on to predict the universal occurrence of this phenomenon of reduction in all plants that reproduce sexually. Concerning the phylogenetic origin of the reduction process Strasburger held that all plants (and animals) were primitively nonsexual and had a constant number of chromosomes. With the development of sexual reproduction the initiation of the process of chromosome reduction avoided the evident disadvantage of repeated doubling of the chromosome number at each sexual fusion. This return from the double number formed in the zygote to the primitive, ancestral number of chromosomes, he believed might occur at any point in the life cycle before the next fertilization. Strasburger then went on to emphasize the advantage of the sexual mode of reproduction, when once acquired, in allowing new combinations of parental strains in the offspring, and the disadvantage it had of producing so small a number of offspring. It is to meet this disadvantage, he suggested, in agreement with Bower, that the zygote of forms like *Coleochæte,* mosses, ferns and seed plants took over the function of multiplying the progeny by a sort of polyembryony, the formation of spores. The spore-bearing generation later in the evolutionary history became ultimately independent of the gametophyte, and at a still later period it not only produced two kinds of spores, but also assumed the care and nutrition of the reduced female plant, arising from the larger of the two kinds of spores. Thus, in Strasburger's view, the primitive nonsexual generation is now represented in the archegoniates by only the sexual phase which has gradually lost its power of asexual multiplication, while the sporophyte is a third, a new, generation arisen by specialization of the zygote. There is in the cormophytes then an antithetic alternation of the two most recently evolved phases of the life cycle, while the only clear trace of the primitive non-sexual phase is found in the halved number of chromosomes, which is reverted to by a process of chromosome reduction, at some point in each life cycle.

In the two decades since this famous pronouncement of Strasburger's was made chromosomes have been counted in the different developmental phases of nearly all groups of plants. These counts have shown that wherever there is sexual fusion there is also, at some other point in the life cycle, a reduction of the double number of chromosomes so formed to the single number characteristic of the gametes. In all cormophytes and many thallophytes this reduction occurs at sporogenesis.

The investigation of the complementary phase of the chromosome behavior, the doubling of the number at fertilization, has during the past two decades also led to extremely interesting results.

The earlier workers on sexual nuclear fusion apparently believed that the paternal and maternal nuclear materials became intimately mingled soon after contact of the nuclear walls. Thus Klebahn (1892) described the chromatin reticula of the two nuclei as gradually merging into one, in *Œdogonium,* and Shaw (1898) described the same process in *Onoclea.* It is true that Guignard (1891) had noted that, in *Lilium* and *Fritillaria,* the male and female reticula remain distinct until the prophase of the first nuclear division of the embryo. Later research, however, showed that the paternal and maternal components remain distinct till much later than this; in fact, that the chromatin elements from the two parents do not really fuse at all during the process of fertilization. On the contrary, it is pretty certain that all through the development of the sporophyte the chromosomes from the two sources retain their identity and individuality.

Thus Blackman (1898) and Ferguson (1901) say that in the fusing nuclei of *Pinus* the two chromatin nets never lose identity, and that at the first mitosis of the embryo each constituent gives rise to its own group of chromosomes. This independence of the two chromatins at fertilization has since been seen in a number of species, and it is now believed to persist throughout the life of the sporophyte. The double number of chromosomes is present at each mitosis of this generation, and they sometimes occur in pairs that are assumed to consist of a paternal and a maternal chromosome each. In certain plants also, according to Overton (1909), Gregoire (1910), Stout (1912) and others, the individuality of the chromosomes of the resting nucleus, postulated by Strasburger in 1894, is morphologically discernible. De Vries (1903) emphasized this fact, that the sporophyte, with its two complete sets of chromosomes, is really two beings in one, by designating it as the "$2X$ generation." This contrasts it at once, in this important characteristic of chromosome number, with the gametophyte or "X generation."

Apparently then no actual fusion of the chromosomes is included in the nuclear union occurring at fertilization. The question at once arising is: where in the life cycle is there any fusion, or intimate union of these inheritance-bearing units? The answer to this question was for some time generally believed to be offered by the phenomena associated with the process of "*synapsis.*" Botanists had for some time noticed and figured the peculiar contraction of the chromatin of the spore mother-cell nucleus occurring just before the chromosomes for the reduction division are formed. Moore (1895) reaffirmed Strasburger's view that, even with the best preservation, the chromatin regularly assumes this condition at sporogenesis, and then only. Moore, therefore, declared this condition to be, not an artifact, as many workers had held, but a natural process, which he named "synapsis." In spite of the insistence by an occasional worker that synapsis is an artifact, the impression of its constancy and peculiarity grew more general at the end of the last century. Then in 1901 Montgomery suggested that it is in this process that the long-delayed union of the paternal and maternal chromatin occurs. Montgomery's conception, that each of the double or bivalent chromosomes, formed on emergence from synapsis, is made up of a paternal and a maternal chromosome, which have in some way been paired up during the synaptic process, came to be rather generally accepted.

Recently, however, a number of workers have dissented vigorously from the view that synapsis is a constant, or a highly significant process. Thus Gregoire (1910), Gates (1911) and Lawson (1912) hold that it does not occur unfailingly at sporogenesis. Lawson says that so much of the separation of the chromatin from the nuclear wall as is not due to artifact is attributable to the more rapid growth of the nuclear wall than of the chromatin. Finally all three agree that such a process is not needed for the pairing of the chromosomes, since, as was observed by Strasburger (1905) and others, the chromosomes may regularly appear in pairs in the vegetative mitoses of the sporophyte. Moreover studies of the vegetative nuclei of the sporophyte, especially by Gregoire and his students, show that their chromosomes are closely connected by adhesions, and by pseudopodium-like strands developed between the viscid chromosomes when the new reticulum is formed after each mitosis. Gates (1911) after reviewing recent work on this point holds that the pairs seen in vegetative mitoses are of a paternal and a maternal chromosome each. He sees no adequate reason for thinking that the association of parental chromosomes at synapsis is any more intimate than that which occurs, as he says, "at or soon after fertilization." He evidently regards the connections between sporophytic chromosomes referred to above as affording ample opportunity for any interchange of material or "influences" between the chromosomes. Gates does not say just when the parental chromosomes are first paired up after fertilization nor give the evidence for this. He fails, also, to explain the fact, upon which practically all workers seem agreed, that the halves of the diploid chromosomes are associated with each other in a more intimate way than the chromosomes of any other mitosis in the life cycle.

VI. ALTERNATION AND CHROMOSOME NUMBERS IN THE ALGÆ, 1896–

We have already seen that an attempt was made in the third quarter of last century to interpret the life histories of certain thallophytes, especially among the algæ, in terms of the alternating generations discovered by Hofmeister among the archegoniates. The basis of comparison was the occurrence of a sort of polyembryony at the germination of the sexually produced oospore of these algæ. There was much uncertainty, however, concerning the real correspondence of phases in the two groups, and even as to whether the alternation was of the same sort in the two groups.

With the promulgation of Strasburger's view (1894) regarding the significance of the reduction of the chromosome number in the life cycle, botanists felt that they would now be able to distinguish the phases of a real alternation of generations wherever chromosomes could be counted. A number of workers therefore followed out cytologically the details of development and conjugation of the sexual cells, and the germination of the zygote in various algæ.

The work of Chmielewski (1890), on *Spirogyra*, and of Klebahn (1891) on desmids showed some indications of a reduction process at the germination of the zygote in these forms, though chromosomes could not be counted. Not till very recently was it demonstrated for one of these, *Spirogyra,* that the chromosome number is actually reduced at this time. Tröndle (1911) has counted chromosomes of *Spirogyra* and finds that there is a real reduction here, and that three of the first four nuclei formed in the zygote degenerate, the fourth remaining as the nucleus of the single embryonic plant formed.

In a study of the green alga, *Coleochæte,* Allen (1905) showed that the chromosome reduction occurs with the beginning of germination of the zygote. Hence the group of zoospore-producing cells, arising from the latter, is not to be regarded as a sporophyte, as had often been maintained. Allen thus eliminated the only ancestral prototype of the bryophyte sporogonium that the antithetic alternationists had been able to discover among the green algæ.

The search among the brown algæ for parallels to the chromosome history of the cormophytes has been much more successful. The first case made out, that of *Fucus,* by Farmer and Williams (1896) and by Strasburger (1897) seemed, it is true, not very illuminating. They found the reduction occurring in the first divisions of the eggs and sperm-producing organs, a point where it occurs in no other green plant. This case of *Fucus,* you will remember, is the one used by Scott (1896) to point a moral, when voicing the generally felt criticism of those botanists who proposed "making the number of chromosomes the criterion by which the two generations are to be distinguished." He says:

I venture to think it premature to rush into inductive reasoning from imperfectly established premises. The case of *Fucus* in which the *Fucus* plant is shown to have the full number of chromosomes goes dead against the idea that the sexual generation (and who could call a *Fucus* plant anything but sexual) necessarily has the reduced number of chromosomes. This fact is indeed a rebuff to deductive morphology.

When, however, Strasburger (1906) and Yamanouchi (1909) followed out the logical trend of the chromosome evidence unreservedly, this life history of *Fucus* became more readily comparable with that of the cormophytes and with those of certain brown and red algæ that had in the meantime been elucidated by Williams and Yamanouchi. From this point of view, elaborated by Yamanouchi, the *Fucus* plant with its $2X$ number of chromosomes is a sporophyte and the reproductive organs arising in its conceptacles are sporangia comparable with those of a seed plant. After the reduction, which occurs at the normal point, *at sporogenesis,* each of the four megaspores, without escaping, gives rise to a gametophyte of two fertile cells or eggs. Each of the four microspores in turn forms a gametophyte, or X generation, of but sixteen cells, each of which is fertile and forms a spermatozoid. It is interesting to note here the similarity which has been pointed out by Strasburger and by Chamberlain of the chromosome cycle of *Fucus* to that of animals. In the latter, from the plant cytologist's point of view, the sexual generation has become reduced to the four haploid nuclei formed at spermatogenesis and oogenesis; and the so-called ovary and spermary are really spore-producing organs of the $2X$ or asexual generation.

In the brown seaweed *Dictyota* the discovery of the chromosome cycle revealed, for the first time in any thallophyte, an alternation that seemed clearly comparable with that of the cormophytes in this respect. Williams (1904) was able to show that the morphologically similar, mature plants of *Dictyota dichotoma* differ not only in that some produce spores only and others male or female reproductive cells only, but also that the nuclei of the former have twice as many chromosomes as those of the sexual plants. He found also that the number of chromosomes is reduced at tetraspore formation and held that all this cytological evidence indicated the alternation of the sexual and the tetrasporic plants. The doubts of conservative botanists regarding the regular and necessary sequence of these haploid and diploid plants were dissipated when Hoyt (1910) raised fruiting tetrasporic plants from eggs and mature sexual plants from tetraspores. Hoyt thus demonstrated, for the first time by cultures in any alga, the identity of this alternation with that of the cormophytes. Yamanouchi (1911 and 1913) has demonstrated, cytologically and in part by cultures, the occurrence of an exactly similar type of alternation in the brown algæ *Cutleria* and *Zanardinia,* the life cycle of *Cutleria* seeming peculiarly like that of the cormophytes because the two generations differ not only in chromatin content, but also in structure.

In the red seaweeds also the use of cytological methods and the determination of chromosome numbers has given a series of very suggestive, though not as yet easily interpreted, results. Oltmanns (1898) showed that the nucleus of the carpospore is a direct descendant of the diploid oospore nucleus. Wolfe (1904) decided that in *Nemalion,* a species that does not form tetraspores, the reduction occurs at the budding out of the carpospores from the mass of cells arising by division of the fertilized egg. He therefore follows Oltmanns in regarding the diploid cell mass mentioned as the sporophyte of this species. In a series of red algæ, which have a tetrasporic phase in the life cycle, Yamanouchi (1906), Lewis (1909) and Svedelius (1911) have demonstrated, cytologically, an alternation of two generations very similar in character to that first found in *Dictyota.* Lewis (1912) later proved conclusively by the use of cultures that the haploid sexual plants arise from tetraspores only, while the diploid fertilized egg gives rise, through the carpospores formed from it, to tetrasporic plants only.

In the interpretation of the phenomena seen in these red algæ Yamanouchi regards the tetrasporic plant as the more primitive phase of the $2X$ generation, and carpospore-formation as a sort of secondarily developed polyembryony for multiplying the progeny from each fertilization. Lewis's view, on the contrary, holds that the tetrasporic plant is, in origin, an early, self-propagative phase of the primitive, haploid, sexual generation. Further he suggests that, in accordance with a general tendency evident in many sexual plants, the process of reduction has here been postponed, and pushed forward from the time of carpospore-formation, where it still occurs in the primitive form *Nemalion,* into this originally haploid tetrasporic plant.

Though no generally accepted interpretation has yet appeared of the somewhat varying chromosome cycles that have now been elucidated in green, brown and red algæ, yet the mass of facts thus far obtained presents an impressive picture of the essential identity of reproductive processes in these plants with those found in the cormophytes. Perhaps the most interesting point noted in making such a comparison is the fact that the type of life cycle among algæ that corresponds most closely with that of an archegoniate, *e. g.,* is the type found in several genera of the brown algæ. The fact may be recalled here also that it was to the gametangia of this group that Davis (1903) finally turned in his search for a prototype of sexual reproductive organs of the bryophytes.

VII. SEXUALITY, CHROMOSOME HISTORY AND ALTERNATION IN THE FUNGI, 1820–

In this group of parasitic or saprophytic thallophytes we shall find as great a variety in the type of reproductive process as in their mode of nutrition at the expense of the hosts beset by them. At the beginning of last century fungi were commonly supposed to arise spontaneously "out of the superfluous moisture of the earth and rotten wood."

Observations had been made long before this, it is true, sufficient to render improbable the spontaneous generation of the fungi then commonly believed in. Thus Micheli (1729) had raised a fungus mycelium from spores. Ehrenberg (1820) did the same and also saw the conjugation of Sporodina. Du Trochet (1834) proved that the mushroom arises from threads of the mycelium in the soil.

The spontaneous generation of even the simplest of these parasitic or saprophytic thallophytes—the bacteria—had been denied by Leewenhoek at the end of the seventeenth century. In one his numerous letters to the Royal Society he denies the spontaneous origin of the animalculæ or bacteria which he found in the mouth. These he found present even in the mouths of ladies who clean their teeth carefully! He insists that these organisms are like those he had found in pools of water, and then goes on to say, in a paragraph that reads like a modern health commissioner's report:

> Now when people wash their beer mugs and drinking cups in the water from ponds and streams, who can tell how many of these animalculæ may stick to the sides of the glass and thus get into the mouth.

The hazy or bizarre beliefs concerning the occurrence and the mode of reproduction in the fungi, current at the middle of last century, were dispelled by the studies of a group of able investigators early in the second half of the century. First came the splendid work of the brothers Tulasne (1847–1854) on the smuts and rusts, and their discovery of the oogonium of *Peronospora*. Pringsheim in 1857 studied the sequence in development of the zoosporangia and oogonia of the water moulds. Then came the researches of that master mycologist, Anton de Bary, on the reproductive structures of *Peronospora* (1861), of *Pyronema* and *Sphærotheca* (1863), and on the life histories of the rusts, 1853 and 1865. The results of his own work and that of his students Woronin and Janczewski convinced de Bary that, in the Ascomycetes, as well as in the phycomycetous *Peronosporas*, the contents of an oogonium is fertilized by the escape into it of the living contents of the antheridial tube that grows beside it.

In the seventies and eighties a vast number of detailed observations concerning reproductive processes in the fungi were accumulated by many observers, led especially by de Bary's student, Brefeld. One outcome of this work which concerns our particular problem, was the insistent, though unconvincing denial by Brefeld of the sexuality of the ascomycetes.

In the last decade of the nineteenth century, with the application of new methods of fixing, sectioning and staining, a new era opened in the study of sexuality in the fungi, an era in which American workers have played a prominent part from the beginning.

As early as 1886 Rosenvinge had succeeded in staining the many nuclei of the mycelial cells of toadstools, also the primary basidium nucleus, and the four spore nuclei arising from this.

Humphrey (1892) and Hartog (1895) followed the history of the nuclei in the antheridium and oogonium of *Saprolegnia*, by the use of stained sections, and concluded, as de Bary had done, that there is no fertilization in these forms. Not until the work of Trow (1904) and Claussen (1908) was it proven that the antheridium of these water molds is sometimes functional, and not always vestigial as de Bary (1881) and Humphrey had thought.

The earliest cytological work on the ascomycetes, after the detection of their nuclei by Schmitz, was that of Dangeard (1894). He described and figured a fusion of two nuclei in the ascus of *Exoascus,* of *Peziza,* of the truffle and others. The source of the two fusing nuclei Dangeard did not trace back farther than the subterminal cell of a hooked hypha from which the ascus arises in *Peziza* and others. The ascus with its fusion nucleus he regarded as an oospore.

In 1895 there was announced from Strasburger's famous laboratory at Bonn a discovery which seemed at one stroke to settle the dispute between de Bary and Brefeld, and to definitely demonstrate the occurrence of a sexual nuclear fusion in the sexual organs seen by de Bary. In that year Harper showed that out of the opened antheridial tube of the hop mildew, *Sphærotheca,* a male nucleus passes into the oogonium and fuses with its nucleus. The whole behavior of antheridium and oogonium and their contents had every aspect of a real sexual process, as de Bary had asserted in 1863. What made Harper's discovery still more significant was the determination of the fate of the fusion nucleus in relation to the nuclei of the ascus and spores. Harper found that one of the row of 5 or 6 cells resulting from the division of the fertilized oogonium has two nuclei. These two descendants of the diploid nucleus, formed at fertilization, afterward fuse, and the cell containing them swells to form the single ascus of this species. This, presumably tetraploid, fusion nucleus of the ascus then grows and divides three times to give the eight spore nuclei. In the following year or two Harper (1896–97) demonstrated a sexual fusion of the same type at the initiation of the fruits of another mildew, *Erysibe,* and of the saucer fungus, *Ascobolus.* The *numerous* asci of these forms all arise from binucleate branches of the binucleate, subterminal cell of the fertilized oogonium. Each ascus is at first binucleate, but later, as had been seen by Dangeard (1894), the two fuse and then by division the eight spore nuclei are formed as in *Sphærotheca.*

In the course of the following decade Harper reported the occurrence of two nuclear fusions, like those of *Sphærotheca,* and at the same points in the life cycle, in the mildews *Erysibe* (1896) and *Phyllactinia* (1905), and in the saucer fungus *Pyronema* (1900). Moreover, he found in *Phyllactinia* a synapsis and evidences of a double reduction of the chromosome number in the divisions of the presumably tetraploid, fusion nucleus of the ascus. *Pyronema* proved interesting also in having multinucleate gametes, such as were at this time being studied by Stevens in the white rust, *Albugo.* Harper believed that many pairs of male and female nuclei fuse in the oogonium of *Pyronema.*

As the outcome of this whole series of studies by Harper it seemed clear that there is in many ascomycetes an alternation of a haploid generation, the vegetative mycelium, with a diploid generation, the fertilized oogonium and the ascus-forming hyphæ arising from it. The second fusion, in the ascus, was regarded as a nutritive phenomenon to provide a nucleus adequate in size for the organization of the relatively huge ascus.

At the opening of the century the observations of a number of workers on the simpler ascomycetes, *e. g.,* by Juel (1902), Barker (1902), seemed to establish the occurrence of a nuclear fusion in the oogonium in these forms also. This, with Harper's, work made it seem probable that this fusion is a frequent phenomenon throughout the ascomycetes in general.

The researches of certain other cytological workers, however, convinced them that no fusion of nuclei really occurs in the oogonium of the ascomycetes studied by them. Thus Dangeard (1897 and 1907), working on *Sphærotheca* and *Erysibe,* found no fusion except that in the ascus. Claussen (1907) and Brown (1909) could find no other in the varieties of *Pyronema* studied by them. Both workers find paired nuclei associated in the ascogenous hyphæ and finally in the young ascus. Claussen, therefore, regards the fusion in the young ascus as a fusion of descendants of the sexual nuclei that were *brought together* in the oogonium but did not fuse there. In other words, he thinks it a real sexual fusion which has been deferred. Brown, on the other hand, says that in his plant no antheridial nuclei are concerned, since the antheridium never reaches the oogonium. He therefore regards the fusion of pairs of nuclei, derived from the oogonium, which occurs in the ascus, as one that serves as a substitute for the sexual fusion that primitively occurred in the oogonium. Brown's view is supported further by his work on *Lachnea* (1911), and by Faull's recent work (1912) on certain *Laboulbenias.*

If this view of Brown's be accepted it implies that the original diploid condition of the cells of the sporophyte has been altogether eliminated, except for the brief uninucleate stage of the ascus. In spite of this, however, the whole structure and development of the original $2X$ generation, from fertilized oogonium to mature fruit and ascus, has been retained. This same normal type of vegetative structure, in spite of an abnormal chromosome number, has been demonstrated in gametophyte and sporophyte of aposporous and apogamous mosses and ferns. It is implied also in Lewis's suggestion that, in the red seaweeds, the reduction has been postponed from its original location at carpospore-formation over into the primitively haploid tetrasporic phase of the next generation.

Still other recent work on the ascomycetes, however, supports Harper's view that a double fusion frequently occurs in the ascomycetes. Thus Blackman and Fraser (1906), Fraser (1907–08) and Fraser and Brooks (1909), find evidence of several degrees of loss of function of the antheridia in the different species of the cup fungi *Lachnea* and *Humaria.* In those cases where no antheridial nuclei are discharged into the oogonium, nuclei of this organ itself are believed, by these workers, to fuse in pairs within it. The later fusion in the ascus, which they find in common with all workers, they regard as a nutritive phenomenon.

Until toward the end of last century the basidiomycetes were generally assumed not to be sexual. At least no sexual organs had been described for them, with the exception of the spermagonia and æcidia of the plant rusts. These had been called male and female organs, respectively, by Meyen, before the middle of the century. The very first nuclear studies of the rusts and toadstools, however, revealed the occurrence of a nuclear fusion, and at another point in the life history, indications of the complementary process, a reduction, were discovered.

In the case of the rusts Rosen (1892) saw two nuclei in the æcidiospore of certain species. Dangeard and Sapin-Trouffy (1893) reported the occurrence of a nuclear fusion in the teleutospore. Sapin-Trouffy (1896) found that the cells of the æcidium-bearing mycelium are uninucleate up to the very base of the chain of æcidiospores. Maire (1900) first stated clearly the whole nuclear cycle in rusts: Beginning with the binucleate æcidiospore there follows, in the wheat rust, *e. g.*, the uredo, or rust stage which has a binucleate mycelium and forms binucleate uredospores for several generations. The two nuclei of the young teleutospore, finally formed on this mycelium, fuse as the spore matures. The two divisions of this fusion nucleus in the promycelium give rise to the four nuclei of the four sporidia which germinate to the uninucleate cluster cup mycelium on the barberry. Maire saw in this life history a real alternation of generations, the gametophyte or X generation beginning with the sporidium, the sporophyte or $2X$ generation, with the mother cell of the æcidiospore chain.

Blackman (1904) and Christman (1905) discovered the origin of the binucleate condition of this mother-cell in species of *Phragmidium*. It there arises by the migration of a nucleus from one cell into another, or by the fusion of the cytoplasm of two cells to form the mother-cell of the spore chain. The two nuclei thus brought together divide simultaneously or conjugately, each contributing a nucleus to the first and to each succeeding spore. This conjugate division of the paired nuclei and their descendants was shown to occur all through the uredo generation up to the formation of the young teleutospore. In the interpretation of their discoveries Blackman and Christman differ more widely than in the facts reported. The former reasserts the surmise of Meyen and believes the basal cells of the spore chain are oogonia which were primitively fertilized by the now functionless spermatia, or pycnospores, produced in separate organs on the barberry leaf. Christman, on the contrary, regards the fusing cells at the base of the æcidium as the primitive, undifferentiated sexual organs of these fungi. He holds that male and female organs have never become differentiated in this group, and thinks that the spermatia are, or were, propagative cells of the X generation.

The observations of many workers on the smuts and on the toadstools have shown the frequent occurrence in them of an association of nuclei and the final fusion of two nuclei in the chlamydospore or the basidium. The time and mode of association of the fusing nuclei, or of their progenitors, are very different in different forms. The fusion, and what appears to be the reduction divisions are, however, constant in location in each species, and are always closely associated. Thus, *e. g.*, the nuclear fusion in the smuts often occurs in the chlamydospore, according to Dangeard (1893) and Rawitscher (1912), and reduction evidently follows immediately in the next developmental phase, when this spore germinates to form the sporidia. In the toadstools, according to Wager (1893), Dangeard (1894), Harper (1902), Nichols (1904) and Levine (1913), the fusion of nuclei occurs in the basidium, and the reduction at the very next division of this fusion product, when the four spore nuclei are formed.

The striking uniformity with which the apparent reduction occurs in all basidiomycetes, at the time of formation of the sporidia or basidiospores, affords good evidence that this type of spore-formation is a long-established one, common to the whole group. It thus supports Brefeld's view that the promycelium of the smuts and rusts is homologous with the basidium of the higher forms. That the point in the life cycle where the associated nuclei finally fuse is the point at which it occurred in the earliest basidiomycetes, is not so clear. The modes of bringing about the first association of the paired nuclei are so varied that it is difficult to detect any clearly ancestral type among them all. The structures concerned with this process in the æcidium-forming rusts certainly seem most readily comparable with the reproductive organs of other thallophytes. It seems probable that the occurrence of fusion at the same point in all forms is due to its being postponed in all forms, as long as it could be without being pushed over into another phase of the life cycle.

It would be instructive to spend another half hour, as we can not do here, in considering those peculiar short cuts in reproduction known as apogamy and apospory. These phenomena are so patently secondary, and so relatively infrequent, that they can not be looked to for evidence of fundamental importance concerning the history or the significance of the essential sexual process itself. Their study has, however, served to correct certain false assumptions concerning the relation between the difference in chromosome number, and the difference in structure, of the two generations. For example, the apogamous production, by *Nephrodium molle*, of a normal fern sporophyte with the X number of chromosomes, demonstrates, as no other kind of evidence could, that de Vries was right in regarding the normal sporophyte as really two beings in one. Incidentally too, such phenomena suggest how comparatively unimportant it is, for the structure of the plant, in what manner, and at what point in the life history, the association of the $2X$ number of chromosomes is brought about.

CONCLUSION

In our rapid glance at the progress made in the study of this problem, during twenty centuries, we have seen how for eighteen centuries men attempted to solve the problem by recourse to philosophical reasoning, without the aid of detailed observation or experiment. Then, in less than two centuries, by the use of these means, Camerarius proved that pollination is a necessary condition of seed-formation; Koelreuter demonstrated that characters from both parents appear in hybrid offspring; Amici, Schmitz and Strasburger showed how the mingling of parental qualities is made possible by the approximation and fusion of parental protoplasms and nuclei.

The sexuality which was first suspected, and first experimentally proven, in the seed-plants, has now been demonstrated in all groups of plants save the bacteria and their allies. The primary feature of the process, the union of two parental nuclei, is the same in all. The method of bringing together the two nuclei varies widely, this variation sometimes involving even the complete disappearance of externally recognizable sexual organs. During the evolution of plants old methods of accomplishing the approximation of the nuclei have been discarded, and new methods have arisen. In the latter case a fusion of nuclei of closer kinship has often been substituted for the primitive one of more distantly related nuclei. This seems evidently the case, for example in the apogamous ascomycetes, perhaps also in the basidiomycetes, and surely so in the cases of nuclear fusion in the prothallia and in the sporangia of apogamous ferns.

In the process of fertilization, as we understand it at present, there are brought together two distinct sets of chromosomes, which in the nuclear divisions of the sporophyte, or $2X$ generation, are often found associated in pairs. The exact manner in which these chromosomes become paired, and the possibility of their attaining any more intimate association, either in the resting reticulum or in synapsis, are not yet definitely determined. If, as is indicated by Mendelian phenomena, and as is demonstrated cytologically to the satisfaction of many workers, there is no loss of identity of the chromosomes in the sporophyte, then there is no very *significant* fusion at any point in the life cycle in consequence of the sexual process. Members of the two sets of chromosomes may be interchanged or shuffled, probably at synapsis, and thus new sets or combinations be formed in the haploid nuclei at reduction. These new combinations, however, are still made up of the same discrete individual chromosomes.

The essence of the sexual process then, as far as yet morphologically demonstrated, consists not of a real fusion, but merely of a temporary association, followed by a reassortment at sporogenesis, of those ultimate, inheritance-bearing units—the chromosomes.

Duncan S. Johnson

Baltimore, Md.

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DR. J. J. RIVERS

WITH the death of this aged naturalist in southern California last December, one of the last links joining the present with the beginning of modern science has been broken. Born in England in the early twenties of the last