Extraordinary sexual phenomena in plants[1]

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The usual phenomena of sexuality may be classified under a few definite terms as: primary and secondary sexual states and characters; specific attraction and fusion of gametes (fertilization); specific attraction, pairing, and segregation of diploid chromosome complements (reduction division); diploid and haploid conditions; the influence of secondary sexual states in producing sexual dimorphisms; the time of sex-determination and sex-reversal; and the distribution of sexual states in the individual as represented by unisexual and hermaphroditic individuals and monosporangiate (diecious) and bisporangiate individuals of various types and degrees. All of these conditions are involved in the usual expressions of sexuality in the course of the ontogenetic and phylogenetic developments. There are also various abnormal peculiarities which are widespread in both the plant and animal kingdoms which must have a prominent place in any comprehensive study of sexuality; among which may be mentioned: self and cross sterility; specific compatibilities and incompatibilities; and the several types of parthenogenetic developments in which cells, after male or female sexualization, return either to a secondary sexual state or to a neutral vegetative condition, new individuals being produced without a union with a cell of the opposite sexual state.

In the plant kingdom a number of extraordinary conditions have evolved which tend to throw considerable light on the problem of the nature of sex, and it is the purpose of this paper to catalog some of the more common and well-known cases, in order that they may be brought into the general discussion of the subject which at present has such a prominent place in biological thought.

When we think of sexuality as + (female) and − (male) conditions we are apt to attribute definite differential qualities to the two conditions, and such may really exist, but, on the other hand, the differences may be only due to degrees of in-

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tensity of development of a common condition. In *Spirogyra* and other conjugates, as shown by various investigators, three types of special reproductive cells may be produced—aplanospores, zygospores, and parthenospores. Both the plus and minus cells may produce parthenospores. In organisms with more highly differentiated gametes the female cell alone retains the capacity for parthenogenetic development. This is probably because of its much lower metabolism as indicated by its lower catalase activity, its food-storing ability, etc. In the higher Spirogyras, the entire filament is normally determined as male or female at the time of conjugation, but as various workers have shown, and as is easily observed in certain species, the cells of a filament may react as + (female) toward one filament and as − (male) toward another. This becomes apparent when a filament is parallel with another filament for only a part of its length and comes to be parallel with a third filament for the remaining part. This suggests that possibly in some stages of development, at least, male and female are but relative terms and + and − only different levels or degrees of the same fundamental condition. Various cases have also been reported where each of two conjugating filaments reacts in one part as female and in another as male toward the other. All such phenomena are decidedly illuminating when it is remembered that these filaments are haploids and have originated from a reduction spore.

The Oedogoniaceae present several remarkable conditions in the evolution of their sexuality. Only one phase of the subject will be considered here. In both *Oedogonium* and *Bulbochaete*, certain species produce so-called dwarf males. Among the species which produce filaments with oospheres and androspores, and dwarf males from the androspores, may be mentioned *Oe. Borisianum* (Le Cl.) Wittr., *Oe. nebraskense* Ohashi, and *Oe. Wolleanum* Wittr. The haploid individual coming from the reduction spore, instead of producing oospheres and spermatozoids, like a normal hermaphroditic *Oedogonium*, produces, as stated above, oospheres and androspores. The androspores must be regarded as derived phylogenetically from original spermatozoids. The egg cell or oosphere is usually situated above a sister cell which is usually called a suffultory cell. This cell apparently has some female qualities. The androspores show a partial primary sexual state and are attracted to the oogonium or to the suffultory cell. In the species mentioned they usually settle down on the suffultory cell. In some species of *Oedogonium* and *Bulbochaete* they mostly settle upon the oogonium itself. Thus these androspores plainly show a partial primary sexual state. They react toward and are attracted by the suffultory cell or the oogonium, but at this point the ordinary primary properties disappear or cease to act, and through parthenogenetic development a dwarf male filament is produced as an epiphyte, which finally furnishes the functional spermatozoids and these not only have attractive properties but also the power of fusing with the female gamete. The dwarf male, of course, shows decided secondary sexual dimorphism, not only because its cells are in the secondary male state, but also because it originated parthenogenetically from a cell which had developed at least a partial primary male state, which has a decided effect on its functional activity at the very beginning. The Oedogoniaceae are probably the highest plants in which parthenogenetic male development is possible. In plants higher in the scale, the spermatozoid is apparently too extremely differentiated morphologically and physiologically to be able to germinate; so parthenogensis is known only in the female gamete.

In the Oedogoniaceae then, we have a series of unusual sexual phenomena, all coming from a single haploid spore, the whole series of sexual changes and peculiarities, both normal and abnormal, being produced without a change of chromosome complement. Recently Goldschmidt and others have attempted to develop a theory of sexuality based on the idea of a balance of genes. Anyone can see that a 'balance of genes' is not involved in the matter, since the same genes and the same chromosome complement is maintained from beginning to end. The original haploid filament exhibits a neutral state, secondary female and primary female states, partial primary male state and perhaps a partial primary female state in the suffultory cell, while the dwarf male with the same hereditary complex, with the original complement of chromosomes, shows only a secondary male state and a primary male state. The same 'balance of genes' has produced practically every kind of sex reaction known. The change from one reaction to another is not caused by a change in the balance of genes. It will also be noted that when the cells are in a neutral vegetative state a very different filament is produced from that produced when the cells are in the secondary male state. If a filament could be developed with the secondary female state it would probably show a trimorphism with the other two.

The higher fungi, including both ascomycetous and basidiomycetous groups, have developed a very unusual and remarkable sexual condition. These plants must be regarded as having evolved from the condition of a simple haploid sexual cycle. In this condition the gametes fuse to form the zygote and at the germination of the zygote reduction takes place, giving rise to haploid spores from which the haploid individual is developed, as in the Conjugatae, the simpler *Oedogonia*, and most of the other green algae. In these higher fungi a haploid mycelium is the normal initial stage with uninucleate cells. When sexual reproduction begins, two cells become sexualized as + and − and conjugate directly, as shown by various investigators, a pore being developed between the fused cell walls and the minus determined protoplast passes over into the plus determined cell. However, no fusion of the nuclei takes place. The pair of haploid nuclei must, however, retain some degree of a primary sexual state, since they can so influence each other that they normally divide simultaneously and each new cell apparently receives a pair of daughter nuclei. Thus a mycelium is developed with binucleate cells. In the Ascomycetae this conjugate phase is usually not extensive, but in the Basidiomycetae and Teliosporae an extensive binucleate mycelium may be developed. In the rusts, as in *Puccinia graminis* Pers., the binucleate phase at first produces aeciospores, which may be regarded as simply a special kind of conidia. These aeciospores each have a pair of nuclei and propagate the rust on the wheat plant, developing a conjugate phase or generation. In all Ascomycetae, Teliosporae, and Basidiomycetae, apparently, a cell is finally produced—the initial ascus cell, teliospore, or basidium—in which the pair of nuclei develop the complete primary sexual states and immediately fuse, giving rise to a normal diploid zygote. This zygote nucleus, on dividing, undergoes the reduction division, as is usual in plants with a simple, haploid, sexual cycle, and the resulting ascospores or basidiospores are haploid again with a single nucleus.

In the Basidiomycetae, the binucleate phase has certain interesting complications, in the development of clamp connections, which seem to have some relation to a + and − condition of the conjugate nuclei.

In this case, the sexualization of the cells, which should end in perfect gametes with primary sexual states, apparently stops short of the complete process, and only partial primary sexual states are developed. The sexualization, however, goes one step further than is the case in the androspores of *Oedogonoium* and *Bulbochaete*, so that there is not only an attractive property and reaction toward the opposite sexual state, but an actual penetration of the one protoplast into the other. The ability to fuse is either absent or is neutralized in the presence of the opposite sexual state.

In certain Basidiomycetae various workers have apparently found a number of compatible and incompatible strains within the species, which seem to be involved in the sexual interactions of certain heterothallic mycelia. If there are numerous strains in some of these species of Agarics, as we know them in various species of rusts and smuts, it may be possible that a careful study of them would throw some light on the relation of hereditary strains to sexual compatibility and incompatibility. The whole group needs careful study in this respect, not only of the phenomena themselves, but especially from the standpoint of correct interpretation, which at present seems to be following the fantastic lines developed in connection with sexual phenomena in the higher plants and animals. Apparently sex-reversal is an easily induced process in many of the heterothallic species, since under artificial culture the heterothallic or unisexual mycelia pass over spontaneously to the homothallic or hermaphroditic condition after a time, as found by several investigators, more especially by Vandendries in *Coprinus radians* (Desm.) Fr., and by Miss Newton in *Coprinus Rostrupianus* Hansen.

The conjugate condition is then to be regarded as an intercalated phase, brought about, in the phylogeny of the group, by a mutative change which interfered with the complete primary sexualization, for the time being, of the gametes. But by a parthenogenetic vegetative growth a binucleate phase or generation was produced which shows a succession of evolutionary advances in the various ascomycetous and basidiomycetous groups. With the complete primary sexualization of the paired nuclei the conjugate phase disappears in the formation of the diploid cell or zygote.

Thus we must recognize three general types of sexual states—the secondary sexual state, the partial primary sexual state, and the complete primary sexual state. The partial primary sexual state shows two recognizable phases or conditions; first, primary sexualization to the extent that only attraction toward the opposite state is shown but no fusion whatever, as in the androspores of *Oedogonium* and *Bulbachaete;* second, primary sexualization to the extent that not only attractive properties are present to such a degree that cytoplasmic fusion is accomplished, but also association of the + and − nuclei, without nuclear fusion, however. Such partial sexual states may perhaps also arise in the sexualization of the synaptic chromosomes and become a prolific source of chromosome disturbances and hereditary irregularities.

When a cell is in the secondary sexual state, its hereditary expression and activity are influenced so that sexual dimorphisms are produced. When a cell is in the partial primary sexual state, attractive properties of various degrees are developed, but these do not lead to the fusion of the sexual nuclei, and in the lowest condition not even to cytoplasmic fusion. When a cell is in the complete primary sexual state, there is not only attraction and reaction toward the opposite sexual state, but, if nothing interferes, complete fusion of the two nuclei is accomplished. The fusion of the nuclei brings about a neutral condition, or a secondary male or female state may follow. In some cases in the higher plants at least, as will be shown below, fusion of nuclei may be followed by a further primary sexualization immediately. In all homosporous plants and in all heterosporous species except the diecious ones, fusion of the gametes ends definitely in a neutral vegetative state, the so-called nonsexual sporophyte. The three types or phases of sexualization are developed both as male or as female conditions and have no direct bearing on the differential involved in comparing these conditions with each other as + and − manifestations of unknown physical phenomena.

Sex reversal has been found to be rather common in the unisexual gametophytes of certain homosporous plants and also in the monecious and diecious species of the heterosporous groups. But the sexual condition and differentiation of the gametophytes of heterosporous plants seems to be very extremely fixed. The sexual state handed over to them from the sporophyte seems to become so extreme and the sexual dimorphism so great that we ordinarily think no sex reversal in such an individual could be possible, and this is probably true so far as the male gametophyte is concerned. But there are certain cases in the gymnosperms, and a very general phenomenon in the angiosperms, which clearly show that cells from the female gametophyte may develop + and − relations and react toward each other as female and male, in other words develop complete primary female and male states, which condition brings about fusion of the two types of nuclei.

In the gymnosperms, the archegonium develops an egg and a ventral canal cell. The nuclei are sisters. Usually the ventral canal cell disorganizes promptly. There are sporadic cases, however, in which the ventral canal nucleus does not degenerate but fuses with the egg nucleus. Such fusions have been observed in several genera, especially in *Pinus* and *Picea*. The ventral canal cell in its degenerative process must arrive at a neutral condition and if the degenerative process is arrested it may pass over to the − or male state and fusion with the + egg nucleus is the inevitable result. Here then we have a case in which a cell of a highly differentiated female gametophyte, a sister cell of the egg itself, has its sexual state reversed to a primary male state. It must not be forgotten that these cells come from a haploid individual, so that there has been no reduction of chromosomes and no shifting of hereditary potentialities. The change from reproductive female cell to the male condition, is, of course, well known in animals, as in chickens and pigeons. In the animal, however, the change takes place in a diploid individual.

The angiosperms have what may be regarded as the most remarkable phenomena in the plant kingdom in the processes of triple and multiple fusions. There is apparently nothing corresponding to these conditions in any other plant or animal group.

In certain gymnosperms, as reported by Miss Herzfeld for *Ephedra campylopoda* Meyer, one of the two male nuclei fuses with the ventral canal nucleus while the other fuses with the egg nucleus. This simply indicates that in the given case the sister nuclei had both passed from the secondary female state to the primary female state, which condition should be expected occasionally, since the joint-firs represent a group with a much shortened gametophytic ontogeny, and during the phylogenetic history the differentiation process, through which primary sexualization is brought about, has been thrown back continuously into an earlier stage because of an abbreviation of the cell lineage. In so far as these abnormalities involve the primary sexualization of normally secondary sexual cells, they correspond to the condition of triple fusion in the embryo-sac of the angiosperms.

In the usual type of triple fusion, as developed in the Anthophyta, the two so-called polar nuclei of the female gametophyte fuse with each other and with the second sperm from the pollen tube about the same time that fertilization is accomplished through the fusion of the egg nucleus and the first sperm. It must be remembered that the upper polar nucleus is a sister nucleus of the egg nucleus and the lower polar nucleus is a sister of the upper of the three antipodals which according to Porsch's view probably also represents a degenerate egg phylogenetically. Every possible order in the fusion of the three nuclei has been observed, so that the triple fusion is brought about in a variety of ways. Often the polar nuclei have already fused when the pollen-tube enters the embryo-sac and the sperm nucleus unites with the fusion nucleus. This condition has been reported for various Ranunculaceae, *Tricyrtis*, *Datura*, *Silphium*, etc. In other cases the polar nuclei and the second sperm may all be attracted and all fuse together at the same time. In some cases the male nucleus may come in contact with either of the polar nuclei while they are still separate; in some cases the male nucleus fuses with the upper polar first and the lower is then attracted to and fuses with this diploid nucleus; in still other cases the sperm is attracted to the lower polar first. These differences in the order of fusions are explainable on the hypothesis that the polar nuclei are not always sexualized at the same time, so that either the upper or the lower nucleus may attain primary sexual maturity before the other. In either case the polar nucleus will have a + reaction toward the extremely male sperm nucleus. Now all the cells of the embryo sac are originally in the secondary female state which was handed over from the megaspöre and thus always show an enormous secondary sexual dimorphism when compared with those of the male gametophyte. But in case the two polars fuse together first, they have the + and − reaction toward each other. In probably most cases the lower one has developed the minus or male reaction. In *Alisma* and *Sagittaria*, which the writer studied intensively, the lower polar nucleus was always the smaller. In *Sagittaria latifolia* Willd. the upper polar nucleus is by far the largest nucleus in the embryo sac, considerably larger than even the nucleus of the egg. Thus it is evident that the difference in size of the two polars is not entirely due to their positions in the upper and lower ends of the sac but, to a certain extent at least, to some differential condition or state which is influencing their development. This difference in sexual development or reaction of two cells of the female gametophyte may be comparable to the opposite, reactions which take place in a sexually differentiated *Spirogyra* filament, as presented above, where part of the filament may react as + toward one filament and the other part as − toward another filament. We can then properly speak of a reversal in the one or the other polar nucleus from the female state to the male state.

There has been much speculation as to whether this triple fusion should be regarded as a fertilization, since no sporophyte embryo results from it, but a xeniophyte. There is no essential difference in the attraction and fusion of the nuclei in this case from a normal fertilization. There is a mixture of chromosomes which subsequently act as a unity in the formation of nuclei by indirect nuclear division. That an entirely different course of differentiation takes place is due to neither the heredity involved nor to the number of chromosomes. We know that a sporophyte may result from a haploid or triploid nucleus as well as from a diploid one. The difference in the subsequent hereditary expression is due to the positions and functional states of the cells involved.

Now if triple fusion were the only unusual sexual event in the Anthophyta, we might rest content with our simpler ideas of what is involved in the sexualization of a cell. The most remarkable conditions, however, are the multiple fusions of cells of the embryo sacs of such genera as *Peperomia*, *Gunnera*, etc. In *Peperomia* by an added cell division stage, a 16-celled female gametophyte is produced. These 16-celled sacs must be regarded as secondary conditions derived from a normal eight-celled sac, as Johnson has maintained, and the multiple fusions occurring in them as secondary developments from an original triple fusion. Probably the most extreme case is that which Johnson found in *Peperomia hispidula* Dietr. where 14 of the nuclei of the 16-celled embryo sac fuse together to produce the primary endosperm nucleus. There was no evidence of a fusion with the second sperm from the pollen tube. In *Peperomia pellucida* Kunth eight nuclei fuse to form the definitive nucleus. These remarkable nuclear fusions involve primary sexualization with the + and − interactions although other properties may be involved. In various plants, like *Fucus*, the egg attracts many sperms, but the fusion is nevertheless with a single one.

Among the lower plants, there are several classes which show mutual attractions between cells which do not appear to involve the ordinary + and − reactions of sexualized cells. The attraction of the cells to form a plasmodium in the Myxomycetae, the gregarious association of the cells in the pseudoplasmodium of the Myxobacteriales, and the symmetrical associations of cells resulting in the colonies of *Pediastrum* and *Hydrodictyon* are probably due to special physical states or conditions not directly related to sexualization, or if sexualization is involved we must assume for the time being that other, quite distinct states and properties are present at the same time. With our present imperfect knowledge of the nature of such states and properties it will be most expedient to regard such associations as appear in *Hydrodictyon,* etc. as of a different physical order from primary sexual states, although it must be admitted that there are some similarities in the reactions. In the multiple fusions of nuclei in the abnormal embryo sacs additional states and properties may also be developed, as intimated above, beside the usual + and − or male and female primary sexualization. The wonder is that a workable and working nucleus comes out, and it is not surprising that in *Peperomia hispidula* Dietr. the primary endosperm nucleus gives rise to only about 40 endosperm cells.

Whatever hypotheses or explanations of the sexual states and conditions may be attempted, the remarkable sexual phenomena recounted above must be taken into account as well as the conditions which we assume to be normal, otherwise they will be of little or no value in the scientific attempt to solve the mysteries of sexuality. It seems foolish to the writer to attempt theories until we have at least made a study of the phenomena that may possibly have a fundamental bearing on the problem we are attempting to solve. It must also be evident to any one acquainted with even only the ordinary phenomena of sexuality, that the recent genetic speculations which have been put forth with such seemingly great confidence have very little to do with the matter, and their main effect seems to be to teach young biologists certain dogmas that make even the simplest and easiest experimentation on sex control and sex reversal seem impossible.

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