The Periodic Reduction of the number of the Chromosomes in the Life-History of Living Organisms[1].

BY

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THE simplest organisms with which we are acquainted reproduce themselves in only an asexual manner. It would appear that it is only in the lowest organisms that the absence of sexual differentiation is possible, and that this differentiation necessarily accompanies a certain definite degree of organization: it is, in fact, as if this differentiation must manifest itself at a certain stage of phylogenetic evolution in virtue of certain properties possessed by organized matter as such. It is true that many highly organized plants are asexual, but comparative investigation proves that this is due to a gradual loss of sexual differentiation, as in the great group of the Fungi, and doubtless also in the apogamous Ferns.

It appears that the sexual act has always given a powerful impulse to phylogenetic evolution; and that, on the other hand, all advance in development was in abeyance so long as sexual differentiation had not been obtained. From the phylogenetic standpoint, we must assume that all sexually differentiated organisms are descended from asexual organisms. The process of this descent is clearly illustrated in

[1] Translation of a paper communicated to Section D of the British Association, Oxford meeting, August, 1894.

certain Chlorophyceae, in which the sexual act consists in the coalescence of swarming gametes. These gametes are obviously derived from asexual swarm-spores, which they quite resemble except in that they are smaller and often have fewer cilia.

The sexually differentiated plants manifest certain differences in their ontogeny, from which it is possible to infer what was the course along which the phylogenetic differentiation proceeded after sexual differentiation had taken place. The simplest case is that in which the product of fertilization gives rise to an individual similar to those which gave rise to the product of fertilization ; and which closes its own life-history with the development either of sexual organs or of asexual organs homologous with them. This occurs in many Chlorophyceae, where, from the zygospore (the product of the coal-escence of similar gametes) or the oospore (the product of the coalescence of dissimilar spermatozoids and ova), a generation is developed which resembles the preceding and gives rise either to swarm-spores or to sexual cells homologous with them. Generally, any one sexual generation follows after a number of asexual generations, the relation being, however, dependent on external conditions, so that, as Klebs has shown, the development of a sexual or an asexual generation can be determined by the observer. In such cases there is a homogeneous sequence of generations which does not include any other kind of sequence or alternation beyond the development either of asexual reproductive organs or of sexual organs homologous with them. The asexual reproductive organs are especially concerned with the rapid multiplication of the individuals under favourable external conditions; whilst sexual reproduction is of importance in maintaining the existence of the species under circumstances which are unfavourable to the vegetative existence of the individual. At the same time, sexual reproduction ensures certain advantages which arise from the coalescence of distinct sexual cells.

In proportion as the asexual mode of reproduction was replaced by the sexual, the numerical conditions of multiplication were maintained either by the development of a number of oospores, as in certain Fucaceae; or, in addition to the sexual organs, altogether new organs were developed to ensure rapid and vigorous development of new individuals in an asexual manner. This took place in various ways. Either asexual reproductive organs were intercalated in the life-history of the original generation, or an altogether new asexual generation was developed from the product of the sexual act. The independent individualization of these different stages of development of the sexual generation into special organs for vegetative multiplication, or into distinct bionts, was carried out to the highest degree in the Fungi and led to the evolution of the many different reproductive forms occurring, for instance, among the Ascomycetes. These arrangements for asexual reproduction were so efficient in the Fungi that the result was the disappearance of the sexual organs and of sexual reproduction. In the Mosses, on the one hand, and in the Vascular Cryptogams and Phanerogams, on the other, there sprang an altogether new generation from the product of the sexual act, the function of which is to reproduce asexually a large number of individuals. The degree of development attained by this generation differed accordingly as its activity was entirely limited to reproduction, or included also nutritive functions. In the Muscineae, this generation is restricted to the asexual multiplication of the individual, and hence it is, in these plants, the sexual generation in which the thallus has attained cormophytic differentiation into stem and leaf. In the Vascular Cryptogams, the centre of gravity of phylogenetic evolution is transferred to the asexual generation springing from the product of the sexual act: this is the generation which, in these plants, attained and advanced in cormophytic differentiation. In proportion as this evolution took place, the nutritive apparatus of the sexual generation became of less importance; and it became altogether superfluous from the moment when the asexual generation began to provide its spores with the material necessary for the development of the sexual generation. In accordance with the general law which determines the phylogenetic disappearance of organs which have become useless, the vegetative parts of the sexual generation became more and more reduced, until little was left but the reproductive organs themselves: hence the progressive reduction in the prothallium from the Ferns up to the Phanerogams. This reduction culminated in the complete loss of independent existence by the sexual generation, because it had ceased to be able to nourish itself independently, and its becoming enclosed by the asexual generation. In consequence of this enclosure of the sexual in the asexual generation, the advantageous rapid multiplication of individuals which the latter originally effected was lost: in order to compensate for this loss, a large number of seeds were produced in the Phanerogams in place of the numerous spores of the Cryptogams; that is, multiplication is effected now by the products of fertilization instead of by asexual spores.

Alternation of generations is absolutely necessary only in those groups of plants in which the fertilized ovum gives rise to the asexual generation, and the asexual spore to the sexual generation. In all such plants the asexual generation is the product of a sexual act. It is important to draw attention to this fact, since it forms the basis of the views which will be subsequently expounded.

From what has been stated in the foregoing paragraphs, it it is clear that throughout the Plant-Kingdom (as far as sexuality is present), sexual differentiation was preceded by asexuality. On the other hand, it must be clearly apprehended that in all those divisions of the Plant-Kingdom in which a true alternation of generations obtains—that is, a necessary alternation of a sexual with an asexual generation—as in the Muscineae, Vascular Cryptogams, and Phanerogams, the sexual generation is to be regarded as the older and as having arisen from an asexual form. Similarly, comparative investigation teaches that the second generation, developed from the product of a sexual act, must be phylogenetically the younger. The gradual development of this generation from the sexual product of the first generation can be actually traced step by step phylogenetically. The first indication of this development is apparently to be found in the Algae : at least the life-history of *Oedogonium*, *Coleochaete*, and the Florideae, may be interpreted in this sense. In *Oedogonium*, four swarm-spores are formed from the fertilized ovum ; whilst in *Coleochaete* a small multicellular body is developed, from the cells of which swarm-spores are formed : in both cases the swarm-spore gives rise to the first generation. In the Florideae the cystocarp is developed from the fertilized ovum, and the spores of the cystocarp give rise to individuals of the first generation. The Muscineae and the Pteridophyta can readily be traced to the Chlorophyceae : in the Muscineae the fertilized ovum gradually developed into a sporogonium, and, in the Pteridophyta, into a sporangium-bearing cormophytic plant.

In the consideration of the alternation of generations obtaining in all the higher plants, importance is chiefly attached to the sexuality and asexuality of the two alternating generations respectively. But, as a matter of fact, it would be more accurate to lay emphasis on the mode of origin of the two generations : from this point of view the sexual generation would be characterized as the gamogenic or sexually-developed generation.

Our insight into the nature of the process of fertilization was very materially promoted by the discovery, made by Edouard van Beneden [1], that the number of the chromosomes is the same in both the conjugating nuclei. Further investigations established the fact, for both animals and plants, that a reduction to one-half of the number of the chromosomes in the generative nuclei precedes the sexual act, and that, in consequence of the coalescence of the male and female nuclei, the nucleus of the fertilized ovum possesses the number of chromosomes characteristic of a vegetative cell.

Basing myself on the observations which had been made

[1] Rech. sur la maturation de l'œuf, la fécondation, et la division cellulaire, Arch. d. Biol. IV, 1883, p. 403

on plants, I have[1], from the very first, maintained the view that the reduction in the number of chromosomes in the generative nuclei is not effected by the extrusion of some of them during the ripening of the sexual cells, but that, on the contrary, the development of the sexual cells depends on indirect nuclear division with longitudinal splitting of the chromosomes; and these views are also held by many observers as regards animals, and by Guignard[2] as regards plants. Guignard and I[3] established the fact that the number of chromosomes characteristic of the generative nuclei of Angiosperms, is determined, in the one case, in the mother-cells of the pollen, and, in the other case, in the mother-cells of the embryo-sacs. The investigations of the zoologists have also shown that this determination is effected in the mother-cells of the ova and of the spermatozoa of animals, by two successive cell-divisions, which give rise, in the one case, to four spermatozoa, and, in the other, to the ovum and to three so-called polar bodies. Guignard[4] in particular has endeavoured to follow with absolute accuracy all the processes affecting the reduction of the number of the chromosomes in the pollen-sacs and ovules of Lilies. The reduction takes place directly, both in the mother-cells of the pollen and in the mother-cell of the embryo-sac, and in such a manner that the reduced number of chromosomes is at once apparent in the prophase-stage. In all the preceding nuclear divisions, both in the pollen-sac and in the ovule, twenty-four chromosomes are, almost uniformly, visible: the framework of the resting-nucleus of the pollen-mother-cell and of the embryo-

[1] Neue Unters. üb. den Befruchtungsvorgang, 1884, pp. 16, 82: Ueb. Kern- und Zell-Theilung, 1888, p. 232.

[2] Études sur les phenomènes morphol. de la fécondation, Bull. de la Soc. Bot. de France, XXXVI, 1889, p. 106, &c.

[3] Strasburger, Ueb. Kern- und Zell-Theilung, 1888, pp. 51 and 240 ff.: Guignard, loc. cit., p. 105 ff.; and Nouvelles Études sur la Fécondation, Ann. Sci. Nat., Bot., Sér. 7, t. XIV, 1891, p. 246 ff.: compare also, Overton, Beitr. z. Kenntniss der Geschlechtsproducte des *Lilium Martagon*, Festschrift für Kölliker und Naegeli, 1891.

[4] Nouvelles Études, pp. 173, 182.

sac-mother-cell is therefore constructed of twenty-four chromosomes: yet in the next prophase it nevertheless uniformly gives rise to only twelve chromosomes. In connexion with this reduction the nucleus does not undergo any diminution either in size or in mass: on the contrary, those nuclei in which the reduction in the number of the chromosomes takes place are remarkable for their size and for the abundance of chromatin which they contain. I contrasted, also in *Lilium*, the cell which gives rise to the embryo-sac with the mother-cells of the pollen; but it must be borne in mind that in *Lilium*, as in *Tulipa* and *Fritillaria*, this cell develops directly into the embryo-sac without undergoing that division which, in other cases, distinguishes the cell as a mother-cell. Hence these observations left open the possibility that the reduction in the number of the nuclear chromosomes might take place in the young embryo-sac and not in the mother-cell of the embryo-sac. I have, however, succeeded [1] in determining that the reduction of the chromosomes to eight or twelve in the embryo-sac-mother-cells of the ovules of *Allium* and *Helleborus* respectively, takes place before they have undergone their characteristic divisions. Hence the cell of *Lilium*, in which the reduction in the number of the chromosomes takes place, must undoubtedly be regarded as an embryo-sac-mother-cell, the course of development being abbreviated. The successive divisions of this cell, in those cases in which they actually take place, give rise, in addition to the embryo-sac, only to reduced cells which are immediately compressed and absorbed. The idea suggested by Bütschli [2] that the polar bodies of animals may be reduced ova was definitively established by O. Hertwig's [3] comparative investigation of the development of the ova and spermatozoa of the Nematodes. The mother-cell of the egg in animals gives rise, by two successive divi-

[1] Ueb. Kern- und Zell-Theilung, p. 243.

[2] Gedanken üb. die Morphol. Bedeutung der sogenannten Richtungskörper, Biol. Centralbl., IV, p. 5, 1884.

[3] Archiv für mikr. Anat., Bd. 36, 1890.

sions, to four cells, and so does the sperm-mother-cell[1]. But whilst the products of division are, in the latter case, all similar, each developing into a spermatozoon, in the former only one develops into a fertile ovum, the other three are polar bodies, that is, they are merely reduced ova. Whilst in plants a reduction in the number of the chromosomes takes place in an unmistakable manner in the nuclei of the mother-cells of the pollen and of the embryo-sacs, it appears, on the contrary, as if in the Nematodes a doubling of the number of chromosomes took place, in the first instance, in the mother-cells of the ova and spermatozoa. But this increase in the number of the chromosomes is only apparent, for it is dependent upon the fact that, in this case, the chromosomes for the two following divisions are provided by longitudinal splitting before they have begun. The phenomenon is merely one of abbreviation : it is nothing more than the compression into one of the longitudinal splittings of the chromosomes attending two successive divisions. The reduction of the number of the chromosomes to one-half thus only becomes apparent in the spermatozoa, the ovum, and the polar bodies, although, as a matter of fact, it takes place in the mother-cells.

But what is the significance of this reduction in number of the chromosomes in the sexual cells, and of the equality of their number in the male and female cells? The physiological utility of the arrangement readily suggests itself: for were it not so, the number of chromosomes in the nuclei of each generation would be twice as great as in the preceding ; and again, by this means each parent is represented in the offspring by an equal number of chromosomes, and thus equally transmits its hereditary characters. The morphological cause of the reduction in number of the chromosomes and of their equality in number in the sexual cells is, in my opinion, phylogenetic. I look upon these facts as indicating

[1] The literature relating to this subject is cited in my work 'Schwärmsporen, Gameten, pflanzliche Spermatozoiden, das Wesen der Befruchtung,' p. 151.

a return to the original generation from which, after it had attained sexual differentiation, offspring was developed having a double number of chromosomes. Thus the reduction by one-half of the number of the chromosomes in the sexual cells is not the outcome of a gradually evolved process of reduction, but rather it is the reappearance of the primitive number of chromosomes as it existed in the nuclei of the generation in which sexual differentiation first took place. Viewed from this standpoint, many facts become more readily intelligible: for instance, the immediate and sudden occurrence of the reduction, the developmental stage at which it takes place, and the varying length of the interval which separates it from the sexual act.

The number of chromosomes determined in the mother-cells of the pollen of the Angiosperms persists up to the formation of the spermatic nucleus in the pollen-tube. Four divisions are involved in the development of this nucleus: two divisions take place in the mother-cell resulting in the formation of four pollen-grains; then there is the division in the pollen-grain by which the generative and the vegetative cells are respectively formed; and, finally, there is the fourth division, the division into two of the generative cell in the pollen-tube. The number of chromosomes determined in the mother-cell of the embryo-sac persists through a series of divisions, the number of which varies with the species of plant, until it attains functional importance in the ovum. As a rule, the mother-cell of the embryo-sac divides twice, and the lowest of the resulting daughter-cells develops into the embryo-sac. In the embryo-sac three divisions succeed each other before the nucleus of the ovum is formed. In this case five divisions, and not four as in the development of the spermatic nucleus, intervene between the reduction and determination of the number of the chromosomes, on the one hand, and the constitution of the sexually-functional nucleus on the other. That the number of these intervening divisions is not of primary importance, is proved by the fact that the number is not always the same: thus, in *Lilium* and *Tulipa* there are but three; in *Ornithogalum*, *Commelyna*, and species of *Agraphis*, there are four; in yet other cases the number is greater than five, as in *Rosa livida*[1], in which case, it should be mentioned, the moment at which the reduction takes place has yet to be ascertained and thus also the identity of the cell established which functions as the mother-cell of the embryo-sac. In view of these facts, it is not surprising that in *Scilla*[2] and *Ornithogalum* the division of the spermatic nucleus in the pollen-tube may be repeated, so that often four spermatic nuclei are formed instead of two. The attempts which have been made to establish homologies between the individual successive divisions which precede the formation of the nucleus of the ovum, on the one hand, and those which precede the formation of the spermatic nucleus, on the other, are thus shown to be futile: and equally barren is the attempt to establish, on physiological grounds, the necessity for a certain definite number of nuclear divisions, based on the assumption that it is by these successive divisions that the male and female nuclei are brought to the same bulk. The whole process appears in an altogether new light when considered from the point of view that the reduced number of chromosomes in the mother-cells in question is the expression of the original ancestral number of chromosomes existing before the sexually-produced generation had been evolved.

The reduction of the number of the chromosomes in the pollen-mother-cells of Angiosperms, which has been adduced as an example, is therefore not to be regarded as a preparation for the sexual act; it really marks the beginning of the new generation which comes into existence with the primitive number of chromosomes. This primitive generation has, however, undergone great limitation before it attained the reduced ontogeny which it now exhibits in the Angiosperms. In the first place it developed sexual dimorphism, so that it

[1] Strasburger, Angiospermen und Gymnospermen, 1879, p. 14, Taf. IV.

[2] Strasburger, Neue Untersuchungen üb. den Befruchtungsvorgang bei den Phanerogamen, 1884, p. 17.

was represented by two parallel developmental series, a male and a female. The phylogenetic course along which this reduction, as also the development of the dimorphism, proceeded, can be traced backwards.

Overton was the first to point out that, in the Gymnosperms also, the nuclei of the mother-cells of the pollen and of the embryo-sacs contain only half the number of chromosomes as compared with those of the plant developed from the ovum. He had already been rightly led by his researches on *Lilium* to raise the question [1] 'whether the reduction may not take place, in the Vascular Cryptogams and the Mosses, in those cells which are the morphological equivalents of the mother-cells of the pollen and of the embryo-sacs of the Angiosperms ; in other words, whether the reduction does not take place in the mother-cells of the spores, that is, at the point of alternation of the generations.' In the mother-cells of the pollen of *Ceratozamia*, Guignard [2] counted eight chromosomes, and found that this number persisted through the subsequent divisions. Overton [3] found the same number of chromosomes in the developing endosperm in the embryo-sac. Guignard ascertained in *Ceratozamia*, and I in several Conifers [4], that all the nuclear divisions in the mother-cells of the pollen and in the pollen-grains themselves are accompanied by longitudinal splitting of the chromosomes. At the same time I drew attention to the uniformity in the number of the chromosomes in the pollen-grains and ova of the Conifers; and I also suggested the probability that in Gymnosperms also the number of the chromosomes is determined in the mother-cell of the embryo-sac [5]. This last point still remains to be proved, since

[1] Ueb. d. Reduction der Chromosomen in den Kernen der Pflanzen, Viertel-jahrschr. d. naturforsch. Ges. in Zürich, XXXVIII, 1893 ; also previously in Ber. d. Schw. Bot. Ges., Heft III, 1893 ; Jahresber. d. Züricher Bot. Ges., 1892–3, Sitzung von 21. Jan. 1892 ; and Annals of Botany, Vol. vii, No. XXV, March, 1893.

[2] Journal de Botanique, III, 1889, p. 232.

[3] Ueb. d. Reduction der Chromosomen, &c.

[4] Guignard, l. c. ; Strasburger, Ueb. das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen, 1892, p. 34.

[5] Loc. cit., p. 35.

Henry H. Dixon[1] found it necessary, in his investigation carried on in the Botanical Institute of the University of Bonn, in the spring of 1893, to confine his attention to ascertaining the reduction by half of the number of chromosomes in the endosperm-tissue of *Pinus sylvestris*. It can, however, hardly be doubted that the reduction takes place in the mother-cell of the embryo-sac as it does in the mother-cell of the pollen. However, without going beyond what is actually ascertained, namely, that the reduction in the number of the chromosomes takes place in the developing endosperm long before the development of the archegonia has begun, there is sufficient evidence to prove that the number of successive divisions which the nuclei with the reduced number of chromosomes have to undergo is altogether different and without relation in the parallel male and female generations. For instance, in *Biota orientalis* only five nuclear divisions intervene between the mother-cell of the pollen and the development of the spermatic nuclei: the pollen-mother-cell divides twice; the pollen-grain divides once, forming the small generative and the larger vegetative cell; the generative cell divides once, and the anterior of the two cells divides in the pollen-tube, forming the two sexually functional cells[2]. With this are to be contrasted the very numerous nuclear divisions which must take place in the embryo-sac of this plant before tissue-formation begins, and, in addition, the number of nuclear divisions which intervene between commencing tissue-formation and the completed development of the archegonium. Dixon[3] counted, in *Pinus sylvestris*, only eight chromosomes in the nuclei of the young endosperm, as also in the ovum at the time of the formation of the canal-cell: on the other hand, I had counted twelve chromosomes in the pollen-grains of the same plant[4]. From renewed investigation made in the

[1] Fertilization of *Pinus sylvestris*, Annals of Botany, Vol. viii, No. XXIX, p. 21, 1894.

[2] Strasburger, Ueb. das Verhalten des Pollens, &c., bei den Gymnospermen, p. 19.

[3] Loc. cit., p. 29 ff.

[4] Ueb. das Verhalten des Pollens, &c., p. 34.

spring of this year, I come to the conclusion that both the pollen-mother-cells and the pollen-grains of *Pinus sylvestris* have only eight chromosomes. The counting of the chromosomes in this material is attended with great difficulty, and is somewhat uncertain, for the limits of the individual chromosomes are not clearly distinguishable, and moreover the chromatic segments in the chromosomes, when division is about to take place, are very distinct and often produce the impression of being independent chromosomes; hence it is easy to conclude that the chromosomes are more numerous than is really the case. The nuclei in the nucellus and in the integuments of the same species of *Pinus* were found by Dixon to contain sixteen chromosomes, and my older preparations clearly show that the dividing nuclei of the developing embryo in the lower end (the morphological apex) of the ovum of *Pinus sylvestris* have more than eight, probably sixteen, chromosomes. They agree, as regards the number of the chromosomes, with the figures of *Picea vulgaris* which I published in 1880[1].

Overton[2] has already drawn attention to the fact that the processes which go on in the spore-mother-cells of the Vascular Cryptogams and the Mosses so closely resemble those by which the reduction in the number of the chromosomes in the mother-cells of the pollen is effected, that their significance is probably the same in both. He found the actual determination of the number of the chromosomes to be attended with great difficulty in the Muscineae on account of the small size of the nuclei, and in the Pteridophyta on account of the large number of the chromosomes. As regards the Pteridophyta, although the number of the chromosomes is considerable in some of them, in others it is not greater than in the Phanerogams: for instance, in *Osmunda regalis* the chromosomes can be easily counted. I ascertained that there are twelve chromosomes in the spore-mother-cells of this plant. The differentiation of them in the

[1] Zellbildung und Zelltheilung, 3e Auflage, Taf. IV.

[2] Loc. cit.; p. 12 of the separate copy.

nucleus of the mother-cell as it leaves the resting stage takes place just as directly as in the pollen-mother-cells of the Phanerogams. It can be easily ascertained that this number persists through the two divisions which result in the formation of the four spores. On the other hand, the nuclei of the archesporial cells, previously to the differentiation of the spore-mother-cells, contain a larger number, probably twice as many or nearly so. This higher number persists, after the differentiation of the spore-mother-cells, in the external tissues of the sporangium. This is shown in the figures published by Dr. J. E. Humphrey[1], who investigated the nuclei of *Osmunda*, with regard to the behaviour of their centrosomes and nuclei, in my Botanical Laboratory last winter. Thus his Fig. 11 shows a mother-cell of *Osmunda regalis* undergoing the first division, and Fig. 12 a mother-cell undergoing the second division; whilst Fig. 10 also shows the division of a tapetal mother-cell. Prothallia, developing from spores sown in a culture-solution, showed twelve chromosomes in all stages, that is, the same number as in the spore-mother-cells. The search for nuclear divisions in developing prothallia requires a great deal of patience, for, so far as my experience goes, they do not take place at any particular time of the day, and hence they are only to be found in isolated cases. My attempts to arrest the nuclear divisions by exposure to low temperatures, or by absence of light, so that they might take place more frequently at appropriate times, led to no result: influences which had proved to be effectual in *Spirogyra* had in this case no effect. All that I could do was to examine a very large number of prothallia which had been fixed in alcohol at various times of the day, and had been stained. I was successful in carrying the counting of the chromosomes up to the commencing development of the antheridia and spermatozoids, and in all cases I found the number to be the same. It is unnecessary to say more than that the processes

[1] Bericht. d. deutsch. bot. Ges., 1894, Heft 5, Taf. VI: see Notes, this number.

which lead up, on the one hand, to the development of the numerous spermatozoids in the antheridium, and, on the other, to the development of the single ovum in the archegonium, differ both in the number and in the succession of the divisions. It is therefore impossible that the divisions taking place in the sexual organs can be of importance in the direction of ensuring equivalence of the sexual cells in preparation for the sexual act. Nor does a process of any kind whatsoever take place by which an equality in number of the chromosomes in the nuclei of the sexual cells is secured : this number is fixed once and for all in the mother-cells of the spores. It may therefore be concluded with regard to *Osmunda regalis*, and doubtless also with regard to all Ferns, that the nuclei of the sexual generation contain only half as many nuclei as do those of the asexual generation. Nor can there be any doubt that in the Ferns the sexual generation is the older: the second arose by progressive phylogenetic differentiation of the product of the sexual act after the first had become sexually differentiated, and hence its double number of chromosomes.

With regard to the Muscineae, the counting of the chromosomes has recently been undertaken by J. Bretland Farmer[1]. He found in *Pallavicinia decipiens*, a Liverwort from the mountains of Ceylon, that the dividing nuclei of the sexual generation (gametophyte) each contain four chromosomes. In the asexual generation (sporophyte) Farmer counted eight chromosomes in the nuclei; and he further ascertained that the mother-cells of the spores have only four chromosomes in their nuclei, so that a reduction by half of the number of the nuclear chromosomes must take place in these cells. The mother-cells of the spores increase considerably in size previously to division, and at the same time become tetrahedrally four-lobed. Between the lobes, septa grow inwards towards the centre of the cavity; and a four-poled spindle is

[1] Studies in Hepaticae : On *Pallavicinia decipiens*, Mitten, Annals of Botany, Vol. viii, No. XXIX, March, 1894.

formed about the nucleus, each pole corresponding in position to a lobe of the cell. The four chromosomes now become differentiated in the nucleus of the spore-mother-cell, and undergo, as Farmer believes himself to be justified in asserting, double longitudinal splitting; four chromosomes then wander into each developing spore: finally, the septa separating the four spores are completed. The same processes take place, according to Farmer, in the spore-mother-cells of *Aneura*. Photographs of the preparations, which I owe to the kindness of the author, bear out the correctness of his statements.

It may appear superfluous to enter upon further speculations how the relations in the number of the chromosomes present themselves in the lower Cryptogams, the Fungi and Algae. I will, however, venture to formulate the problem which has to be solved with reference to these plants, in the hope of giving a stimulus to the necessary research. As a matter of fact, no countings of the chromosomes in the dividing nuclei in the lower Cryptogams have been undertaken: this is due partly to the great difficulty with which counting is attended, and partly to a lack of appreciation of the importance of these countings. The first question to be asked is, whether in these lower Cryptogams, in which a true alternation of diverse sexual and asexual generations does not take place, the number of the chromosomes in the nucleus is at all determinate; and if it be so, whether and when a reduction takes place of the double number of chromosomes resulting from a sexual act. With regard, in the first place, to the question whether or not there is a determinate number of chromosomes in Algae and Fungi, I am inclined to answer it in the affirmative: for I was able to count twelve chromosomes in the nuclear discs of *Spirogyra polyteniata*[1], which number was also determined by J. W. Moll in *Spirogyra crassa*[2]; and I am almost certain of the uniformity in number of the chromosomes in *Trichia fallax*, an organism belonging

[1] Kern- und Zell-Theilung, p. 11: Histol. Beitr. I, 1888.

[2] Observations on Karyokinesis in *Spirogyra*, Verh. d. Kon. Akad. van Wetensch. te Amsterdam, Tweede Sectie, Deel I, No. IX, 1893, p. 29.

to so low a group as the Myxomycetes. I believe that the nuclei of *Trichia fallax* contain each twelve chromosomes: I counted them in my old preparations[1] showing numerous nuclear divisions in developing sporangia. It is true that the nuclei are so small that absolute accuracy in the counting is hardly attainable: still one cannot but be impressed by the remarkably uniform appearance of the nuclear divisions. If, however, the number of the chromosomes be constant in the Myxomycetes, there can be little doubt but that it is so universally: and then it becomes probable that a reduction in the number of the chromosomes must be associated with some definite developmental stage in those of these lower Cryptogams which are sexually differentiated. The assumption that this reduction takes place during the development of the sexual organs is not supported by any direct evidence, and it is contrary to what has been ascertained in the higher Cryptogams. It may be that the reduction follows the sexual act. In all these cases in which the original generation is directly developed from the product of fertilization, the reduction probably takes place during germination: here the zygote is all that represents that developmental stage, the asexual generation, which intervenes in the Muscineae, the Pteridophyta, and the Phanerogams, between fertilization and formation of spore-mother-cells. On the other hand it is possible that, in *Coleochaete*, *Oedogonium*, or the Florideae, the reduction does not take place until the development of the, motile or non-motile, spores with which the product of the sexual act closes its existence; for it is from these spores that the first generation is, in turn, developed.

The constancy of the number of the chromosomes in the nuclei of the sexual cells is doubtless of great importance, for it ensures the equal influence of the two parents in the sexual act: and the act of fertilization is, in all the higher organisms, the centre of gravity of the maintenance and development of the species. In contrast with this is the

[1] Zur Entwicklungsgeschichte der Sporangien von *Trichia fallax*, Bot. Zeitg., 1884, Taf. III, Fig. 6.

fact that the number of the chromosomes in the nuclei of the somatic cells of both the sexual and the asexual generations has been found to vary. But, so far as my experience goes, these variations are always to be observed in the nuclei of cells which are not longer embryonic, like those in an embryo or in a growing-point, but which, on the contrary, are to some extent histologically specialized and are not destined to eventually give rise to reproductive cells. Both Guignard and I have often observed variation in the number of the chromosomes in the cells of the nucellus and integuments of the ovules. The determinate number of chromosomes is still more frequently departed from in nuclei which are definitively excluded from the sphere of reproduction. Thus Guignard[1] found in species of *Lilium* that the lower nucleus in the embryo-sac, from which the antipodal cells are derived, has not twelve chromosomes like the upper nucleus which gives rise to the egg-apparatus, but sixteen, twenty, or even twenty-four chromosomes. The secondary nucleus of the embryo-sac, by the division of which the development of the endosperm is initiated in the Angiosperms, is produced by the fusion of the two (upper and lower) polar nuclei, and must therefore contain as many chromosomes as both the polar nuclei. Hence, in *Lilium*, the nuclei of the endosperm are usually found to contain more than twenty-four chromosomes, although it represents the generation which typically possesses only twelve chromosomes in each nucleus. Some time ago[2] I described the frequent nuclear fusions which gradually take place in the developing endosperm of the Angiosperms when, as the cell-areas are being marked out, each area encloses several nuclei. As regards the Gymnosperms, Dixon was able to ascertain that, in *Pinus sylvestris*, the determinate number of chromosomes was adhered to in the prothallial nuclei of the embryo-sac, until the development of the archegonia: but when the development of the archegonia is initiated, the determinate

[1] Nouvelles Études, p. 187.

[2] See especially, Zellbildung und Zelltheilung, 3. Aufl., 1880, p. 25.

number of chromosomes may be departed from in the other prothallial cells with any possible prejudice to the reproductive processes, and accordingly the number is found to increase, or even to double itself, in the large nuclei of the cells forming the walls of the archegonia [1].

What has just been stated suffices to prove that variations from the determinate number of chromosomes are possible. Similar variations have also been observed among animals, but I will not discuss them as I am not in a position to estimate their significance [2]. Among the examples from the plant-kingdom which have been cited, that of the lower nucleus in the embryo-sac of the Lilies, so carefully studied by Guignard [3], appears to be the most instructive. This nucleus has originally twelve chromosomes, but in the next prophase a larger number can be detected. From this it might naturally be inferred that the reduction in the number of the chromosomes as exhibited in the sexual cells does not call for any phylogenetic explanation, and that it is superfluous to regard it as a reversion to an older condition of things, since a change in the number of the chromosomes may take place quite independently of any such assumption. But the variation is essentially different in the two cases. The change in the number of the chromosomes which is associated with the alternation of generations is accompanied by other deep-seated changes, which can be detected in the altered appearance of the spore-mother-cells. Moreover the change in the number of the chromosomes associated with the alternation of generations gives rise, not to a variable, but to a perfectly constant result, which can only be attributed to phylogenetic causes. The purely vegetative—they may be almost called accidental—variations in the number of the chromosomes within the limits of any one generation, do not otherwise affect the appearance of the nuclei, and the re-

[1] Dixon, loc. cit., p. 32.

[2] Compare especially Valentin Haecker, Ueb. generative und embryonale Mitosen, &c., Arch. f. mikr. Anat., 43, p. 773, 1894.

[3] Loc. cit., p. 187.

sulting number is quite indeterminate. Thus, in the lower nucleus in the embryo-sac of the Lilies, from twelve to twenty-four chromosomes make their appearance in the prophase. The lower nucleus is larger than the upper one, though in other respects similar; and it may be that the increase in number of the chromosomes in the lower nucleus is a definite result of more ample nutrition, and the same influences may be at work in those cases of apogamy in which the number of chromosomes characteristic of the asexual generation is attained in a purely asexual manner. The case of the adventitious development of embryos in Phanerogams is not one that offers any difficulty: for the cells of the nucellar tissue from which the embryos spring already contain as many chromosomes as does the fertilized ovum. But the case of Fern-prothallia from which the cormophytic asexual generation is developed as a bud, is altogether different. The nuclei of the prothallial cells contain only half as many chromosomes as do the cells of the asexual generation: hence it is probable that on the development of the growing-points of the asexual generation, the number of the chromosomes in the nuclei is doubled. Overton, who has already dealt with this problem from the theoretical point of view, is of opinion that it presents no greater difficulty than does parthenogenesis, and he draws attention to the fact that the lower nucleus in the embryo-sac of *Lilium* changes the number of its chromosomes quite independently[1]. Direct observation alone can decide whether the number of chromosomes in the nuclei of an apogamously developed Fern is increased independently; or whether, though I do not regard the suggestion as probable, its nuclei have the same number of chromosomes as those of the prothallium. If the latter be the case, then the development of the spores of these plants is not attended with a reduction in the number of the chromosomes. The assumption that a doubling of the number of the chromosomes takes place, under the influence of correlative processes, in the apogamous development of

[1] Loc. cit., pp. 14, 15, of the separate copy.

a Fern, is supported by the fact that apospory also occurs among Ferns. In certain varieties of *Athyrium*, *Polystichum*, and *Aspidium*, as F. O. Bower has shown[1], fertile prothallia are developed in place of sporangia. It would appear, therefore, that the nuclei of these prothallia must contain twice as many chromosomes as do those of normally developed prothallia; and consequently, since no reduction in the number of the chromosomes occurs in connexion with the development of the sexual cells, these cells would, in this special case, contain twice the normal number of chromosomes. It is, however, more reasonable to assume, until direct observation proves the contrary, that the aposporous development is attended with a correlative reduction in the number of the chromosomes. On similar grounds it is probable that a reduction also attends the development of protonema, eventually bearing sexual plants, which has been induced in the sporogonia of certain Mosses. From the fragments of setae of species of *Hypnum* and *Bryum*, Pringsheim[2] obtained the development of protonema; as did also Stahl[3] with *Ceratodon purpureus*, not only from cells of the seta but also from those of the wall of the capsule. It is probable that in this protonema a reduction of the number of the chromosomes takes place under the influence of correlative processes.

The foregoing phenomena suggest the raising anew of the question as to the continued independent existence of the chromosomes. In the case of plants it may not be considered as settled that, in the resting nucleus, the chromosomes present no free ends. Guignard[4], whose statements are perfectly correct[5], found but a single filament at the beginning of the prophase in the nuclei which he examined.

[1] On Apospory and allied phenomena, Trans. Linn. Soc., Ser. Bot., Vol. ii, Part 14, p. 301, 1887.

[2] Ueb. vegetative Sprossung der Moosfrüchte; Monatsber. d. Berl. Akad. d. Wiss., June 10, 1876.

[3] Ueb. künstlich hervorgerufene Protonemabildung an den Sporogonien der Laubmoose, 1876.

[4] Nouvelles Études, p. 253.

[5] Strasburger, Schwärmsporen, Gameten, &c., Histol. Beitr., Heft IV, 1892, p. 147.

This filament breaks up into a given number of segments simultaneously, not by successive divisions into two: it is on this account that such numbers as twelve are frequently found, numbers which cannot be the result of repeated division into two. The fact that, as a rule, the same number of chromosomes occurs in successive generations of nuclei, suggests the view that though the chromosomes may lose their morphological individuality in the resting nucleus, they do not lose their physiological individuality. The observation of such a series of stages of nuclear division as can be obtained by the laying open of embryo-sacs in which the development of the endosperm is commencing, makes it difficult to resist the impression that it is always the same chromosomes which make their appearance over and over again in the repeated divisions. In the prophase the chromosomes are seen to appear in precisely the same positions as they occupied in the preceding anaphase: and if the picture of the anaphase were proportionately enlarged it would exactly correspond to that of the succeeding prophase. In one word, it must be assumed that the individuality of the chromosomes persists in the resting nucleus, and determines the breaking up of the nuclear filament into the corresponding number of chromosomes in the succeeding prophase. Any change in the number of the chromosomes must be preceded by an alternation, whether increase or diminution, in the number of the chromosomatic individualities. The reduction of the number of the chromosomes by half, at the initiation of the sexual generation, is due to the fusion into one of two chromosomatic individuals, under the influence of causes which, for the present, can only be assumed on phylogenetic grounds. These fusions of chromosomes, at the initiation of the sexual generation, can apparently only take place under certain conditions. They are affected by abnormal internal changes: for instance, the embryonic substance constituting the growing-points of shoots affected with bud-variation often remains sterile; and hybridization frequently induces similar consequences.

My developmental studies on the spermatozoids of plants [1] impressed me with the conviction that the surrender of morphological individuality by no means involves, for these chromosomes, the loss of physiological individuality. It is only on the assumption of the persistence of this physiological individuality that it is possible to account for the fact that a homogeneous filament in the nucleus of a spermatozoid gives rise, in the fertilized ovum, to a predetermined number of chromosomes.

It is established that, in the higher plants, all the nuclear divisions which lead up to the formation of the sexual cells are normally attended by longitudinal splitting of the chromosomes, so that the number of the chromosomes remains the same throughout. There is no such thing, among plants, as nuclear divisions resulting in the reduction by one-half of the number of the chromosomes. Such a conception involves the assumption that the entire, not longitudinally split, chromosomes of the mother-nucleus become separated into two groups, each of which goes to form a daughter-nucleus [2]. If this be so, then each daughter-nucleus must contain only half as many chromosomes as the mother-nucleus; and, in the next generation, each nucleus must contain only half as many chromosomes as a daughter-nucleus: but nothing of the kind can be observed among plants, a fact which has to be taken into account in a consideration of the phenomena of heredity. Among animals too, as is shown by recent researches, the division with reduction taking place in the mother-cells of ovum and spermatozoon is dependent upon previous longitudinal splitting of the chromosomes, and is therefore referable to normal nuclear division: and even were there not sufficient evidence to prove this, the facts are so clearly ascertained with regard to plants, in which the phenomena of heredity and variation are essentially the same

[1] Schwärmsporen, Gameten, &c., p. 145.

[2] Weismann, Ueb. die Zahl der Richtungskörper und ihre Bedeutung für die Vererbung, p. 79, 1894.

as in animals, that all possibility of misinterpretation is excluded, and that their importance cannot be overlooked [1].

Just as the facts ascertained among plants exclude the assumption of nuclear division with reduction, so also do the observations on nuclear division in plants give no support to the view that karyokinesis is ever attended with hereditarily unequal division, and, so far as my information goes, the observations made on animals are likewise unfavourable to this view. Ever since an accurate knowledge of the longitudinal splitting of the chromosomes during nuclear division, and of the equal distribution of the products of this splitting, was attained, I have become more and more firmly convinced that the object of the process is the qualitatively equal division of the chromosomes. Theoretical speculation, which transcends the limits of experience, must start from definitely ascertained facts. Minute investigation of the longitudinal splitting of the chromosomes can but produce the impression of equal division: there is absolutely no foundation in fact for the assumption of unequal division. Hence, from the very beginning, I have taken the standpoint of epigenesis in forming my theoretical interpretation of the facts of development [2]. The only conception of development that I am able to form is that it is a succession of stages, such that each stage determines the conditions for the succeeding stage and inevitably leads on to it. In my opinion, development belongs to the category of correlative processes, and can only be comprehended from this point of view. The cell-nuclei, in whatever part of the body they may be, are and remain

[1] See Boveri, Zellen-Studien, Heft I, 1887, p. 13 ff., 77, and Heft III, 1890, p. 51: also August Brauer, Ueb. das Ei von *Branchippus Grubii* von der Bildung bis zur Ablage, in Anhang zu den Arbeiten der Akad. d. Wiss. zu Berlin, 1892: O. Hertwig also admits the possibility of the reference of division with reduction to normal nuclear division in his Ei- und Samenbildung bei den Nematoden, Arch. f. mikr. Anat., Bd. 36, 1890, pp. 65 ff. of the separate copy.

Also Valentin Haecker, Ueb. Generation und Embryosack-Mitosen, &c., Arch. f. mikr. Anat., Bd. 43, 1894, p. 759: see also my work, Schwärmsporen, Gameten, &c., p. 151.

[2] See, Das Protoplasma und die Reizbarkeit, 1891, pp. 20, 27.

endowed with all the characteristics of the species; but their activity is stimulated in a definite direction by the prevalent conditions. Were this not the case, it would be impossible for the renewed development of organs to take place, as it does, from any part of the plant-body, organs which manifest all the characteristics of the species; nor would it be possible to stimulate certain activities by artificial interference, and to induce this or that manifestation of hereditary capabilities. It is in this way that I account for the influence of those external conditions, for instance, which determine sexual or asexual reproduction in the Algae; as also for the influence of certain substances, formed by the organism itself, which induce the formation of a flower at the growing-point.

I have rejected the view of the hereditarily unequal division of nuclei on the ground that it is contrary to the facts ascertained by direct observation, and I am equally unable to admit that theories of heredity are justified in reconstructing the nucleus with the object of finding in it all the structures which are necessary to them: the only legitimate point of departure is afforded by the actually observed facts of nuclear structure. I consider Weismann's conception of the id [1], as an element in the nucleus which is charged with all the hereditary characteristics of the species, to be felicitous, because it appears to me that it can be supported by direct observation. I regard as ids the discoid segments of the chromosomes, which are all exactly similar in form and structure, and are serially arranged with such remarkable regularity in the chromosomes of nuclei about to divide. Whilst in the resting-stage of the nucleus the substance of each id is distributed, for nutritive purposes, over the elongated nuclear filament, in the prophase the substance segregates to constitute and form a segment of the series. In the id there are represented not only the small chromatin-

[1] Das Keimplasma, eine Theorie der Vererbung, p. 84: p. 60 in the English edition, 1893.

granules previously distributed in the linin-network, but also, and perhaps to the largest extent, the linin-network itself. For it is well known that the staining capacity of the contents of the nucleus increases considerably in the prophase, passing over for the most part into that readily stainable condition which we regard as an increase of the chromatin; in the anaphase the nuclear contents undergo precisely contrary changes. In support of the view that the individual segments present in the chromosomes of nuclei about to divide, possess all the hereditary characteristics of the species and are, in fact, the true ids, may be adduced the results of the microscopic vivisection of unicellular organisms[1]: it has been found, namely, that a fragment of such an organism will regenerate itself to a complete individual if only it contains a portion of the nucleus. Again, I have observed that when, as not infrequently happens during the division of the pollen-mother-cells of *Hemerocallis fulva*, single chromosomes do not enter into the structure of either of the daughter-nuclei, but remain behind in the equatorial plane of the nuclear spindle, small pollen-grains are formed in relation with them. The small chromosome marks itself off from its surroundings, and a proportionate mass of the cytoplasm of the mother-cell is assigned to it[2]. The often very small pollen-grain develops quite normally and shows all the peculiarities of structure characteristic of the species.

The serially arranged ids in the chromosome are, in my opinion, repetitions of each other, for no difference can be detected between them by actual observation. It is possible to go on to assume that they are repetitions which correspond to successive generations, and that they actually represent, as Weismann puts it, ancestral plasm. It is by their simultaneous activity that the constancy of the species is proportionately maintained: for the co-operation of so many ids

[1] See especially A. Gruber, Mikroskopische Vivisection. Ber. d. naturf. Ges. in Freiburg im Br., Bd. VII, Heft I.

[2] Ueb. den Theilungsvorgang der Zellkerne, 1882, p. 20, and Taf. II, Figs. 63–65.

must produce a resultant effect which would be a mean between the individual variations of the successive generations. If, however, in consequence of the repeated union of individuals presenting a similar variation, the number of ids representing this variation be increased, the variation must become permanent.

At each longitudinal splitting of the chromosomes during nuclear division, all the ids are halved and are equally distributed to the succeeding generations of nuclei. The number of the ids would, however, become doubled at each sexual act, were it not for the reduction which takes place at the initiation of each sexual generation. Since this reduction is not due either to extrusion or to an absorption of the chromosomes, at least in plants, the only remaining explanation is that it is due to the fusion in pairs of the ids and therefore also of the chromosomes. In the processes of differentiation which take place in the nucleus of a spore-mother-cell during the prophase, the substance of each pair of ids aggregates into a single id. In this way the idioplasm of many and different ancestors enters into the formation of each individual id. I do not, however, consider that these ancestral plasms exist isolated in the id ; I regard them as completely fused into one. The number of the ids is doubtless, like that of the chromosomes, hereditarily determined : but the numbers in different organisms certainly do not stand in any definite relation to each other, for even closely allied species of plants, which have ids of apparently the same size, sometimes present different numbers of chromosomes : for instance, in the Liliaceae the nuclei of the spore-mother-cells contain, according to the species, 8, 12, 16, or 24 chromosomes. It would appear, therefore, that the number of the chromosomes possesses no deep significance : and do not the two externally indistinguishable varieties of *Ascaris megalocephala*, which have been so fully investigated, differ in that the nuclei of the one contain only half as many chromosomes as do those of the other?

It is now known, and the point has been made especially clear by observations on *Ascaris nigrovenosa*[1], that the chromosomes of the two parents do not lose their independence in connexion with the sexual act. In this *Ascaris* both the sperm-nucleus and the germ-nucleus independently go through the prophases of division, and it is only then that the two sets of chromosomes arrange themselves in the common spindle of the embryonic nucleus. In each subsequent nuclear division each half contains a number of chromosomes identical with that of the combined parental chromosomes in the fertilized ovum. Hence in hybrids the chromosomes of both father and mother continue active; and the behaviour of hybrids shows peculiarities which are very instructive for the comprehension of the phenomena of heredity in the offspring of legitimate unions. Hybrids may exhibit in all their parts a combination of the characters of the two parents; or they may show this only in certain parts, whilst other parts present the distinct characters of one or other of the parents; or they, on the whole, resemble one parent more than the other; or, finally, they may altogether resemble one of the parents. Naudin[2] has drawn attention to the fact that, in some hybrids, the characters of the two parents, instead of being blended, are manifested in patches; this may occur in all parts of the plant, but it is especially marked in flowers and fruits. In such a case the hybrid is a sort of mosaic made up of portions of the two parents. Millardet[3] has recently given an account of hybrids which are more like, or, in the extreme case, exactly resemble either the father or the mother.

The hybrids of mosaic-like constitution may perhaps be adduced as evidence in support of the possibility of hereditarily unequal division of the nucleus; and one of the cases described by Millardet, that of a hybrid Vine, known as the

[1] Edouard van Beneden, Rech. sur la maturation de l'œuf, &c., Taf. XIX, *bis et ter.*

[2] Sur l'hybridité dans les végétaux, Nouv. Arch. du Muséum, I, 1865, pp. 33, 49, 151.

[3] Note sur l'hybridation sans croisement, ou fausse hybridation, Mém. de la Soc. d. Sci. phys. et nat. de Bordeaux, t. IV, sér. 4, 1894.

York-Madeira, would lend itself to this purpose. This hybrid appears to be the offspring of the spontaneous crossing of *Vitis aestivalis* and *V. Labrusca.* On the under side of its leaves it presents not only the sunk stomata of *V. aestivalis* and the projecting stomata of *V. Labrusca,* but also every possible intermediate form of stoma. From these facts the inference might be drawn that the epidermis of the leaf of this plant consists of cells which belong to the type of the father, or to that of the mother, or to an intermediate type. If this be so, the type must be manifested in the individual cells, since the two guard-cells of a stoma are developed from a single mother-cell. If now this case is to be explained by referring it to a difference in the nuclei of these cells, induced by hereditarily unequal division, the attempt might appear plausible enough: but such an assumption would be directly contradicted by those cases in which the hybrid entirely resembles either the father or the mother, cases which have been observed not only in the genus *Vitis,* but also in *Rubus* and *Fragaria.* For in these latter cases it is an inevitable consequence of the theory of hereditarily unequal division, that somewhere in the body of the hybrid there must be a preponderance in favour of the parent which the hybrid does not resemble; but, as a matter of fact, this does not occur. Hence the only possible explanation seems to me to be that the interaction of the chromosomes in the nucleus gives rise to phenomena of interference: in those hybrids which entirely resemble either the father or the mother, it may be assumed that the influence of the chromosomes of the one parent is completely neutralized by that of the chromosomes of the other; whereas in other hybrids some characteristics are weakened, whilst others are strengthened, by interference. Similarly, just as in hybrids, so the offspring of parents of the same species may either combine the characters of the parents, or resemble one or the other more strongly.

Atavistic phenomena clearly prove that the ids whose action is neutralized are not absorbed or otherwise destroyed. A very instructive case illustrating this point is that of the peloric Snapdragon (*Antirrhinum majus*) described by Charles Darwin[1]. The seed produced by the peloric plants when fertilized with their own pollen yielded only peloric individuals: whilst the seed produced by peloric plants fertilized with the pollen of the normal forms yielded only normal plants; and similarly the seed produced by plants of the normal form fertilized with pollen from the peloric form yielded only normal plants. Hence the influence of those chromosomes which induced the peloric condition was, in the two latter cases, neutralized by the chromosomes of the normal form. But the peloric chromosomes were not destroyed, for the descendants of the normal plants of semi-peloric origin were peloric to the extent of one-third.

However peculiar may be the mixture of the parental characters which a hybrid presents, it is repeated in all hybrids having the same origin. But this is not the case with the offspring of hybrids fertilized with their own pollen: on the contrary, the progeny is now remarkable for a high degree of variability. In successive generations resulting from repeated fertilizations with their own pollen, there is a growing tendency to revert to the parental forms. But few hybrids, fertilized with their own pollen, continue to reproduce themselves unchanged, and thus practically constitute new species. Weismann[2] endeavours to account for the variability of the offspring of hybrids by referring it to divisions accompanied with reduction, in the development of the sexual cells: these reducing divisions result in dissimilar products, and the union of the dissimilar products induces variability. This explanation is, however, inadmissible because, as a matter of fact, such divisions do not take place among plants: the causes of the variability of hybrids must be sought elsewhere. They are to be looked for in those processes which lead to

[1] Das Variiren der Thiere und Pflanzen im Zustande der Domestication, Germ. ed., 1868, Bd. II, p. 92: Variation of Animals and Plants under Domestication, ii, pp. 70, 93, 1868.

[2] Das Keimplasma, eine Theorie der Vererbung, 1892, p. 293: Engl. Ed., p. 299.

the reduction of the number of chromosomes in the mother-cells of the spores. Hybrids of similar origin resemble each other, in the first generation, because the chromosomes of both parents persist side by side in all the nuclei of these hybrids, and affect the processes of development in a definite manner. The offspring of these hybrids behave differently, doubtless because there is a fusion of the parental chromosomes and a corresponding reduction in the number of the ids in the process of development of the spore-mother-cells (mother-cells of the pollen and of the embryo-sac). Hence interference becomes more active, and renders possible a difference in the sexual cells ; and the union of these diverse cells in the sexual act is the starting-point of diversity in the progeny. It is conceivable that this diversity is due to the influence of isolated ids, of ids derived from one or other of the original parents, but remaining unfused. It may be further suggested that the continued production of unchanged progeny by hybrids is only possible in those cases in which the chromosomes and the ids of the original parents retain their primitive equivalence even after reduction and fusion have taken place.

The process of reduction of the number of the chromosomes by half takes place, in the Muscineae, Pteridophyta, and Phanerogamia, in the spore-mother-cells, that is, at the close of the generation developed from the fertilized ovum; but in the lower Cryptogams, where the cell produced by the sexual act does not give rise to a definite organism representing the asexual generation, the reduction probably takes place on the germination of this cell. The attempt has been made to give a phylogenetic explanation of this reduction in the number of the chromosomes, and it has been regarded as a reversion of ontogeny to the point of origin. The phenomenon under consideration is essentially that of the return of the most highly organized plants, at the close of their life-cycle, to the unicellular condition: in one word, it is the repetition of phylogeny in ontogeny. This explanation seems to me to be likewise the only one admissible in the case of animals. It is, however, an altogether different question, whether or not the double division which takes place in the mother-cells of the spermatozoa, and of the ova in connexion with the development of the sexual cells in animals, may not be interpreted as indicating the existence of a distinct generation. The remarkable uniformity presented by the double divisions in the development of the sexual cells of animals is unfavourable to the assumption that the product of the double division represents what remains of a once independent sexual generation. Were this the case, it is certain that the reduction would be manifested in different degrees in the various sub-divisions of the animal kingdom. It may, with greater probability, be assumed that, in all those sub-divisions of the animal kingdom, sexual differentiation occurred at the very beginning of phylogenetic development, and that the product phylogenetically developed from the sexual act is the direct continuation of the ontogeny of the individual. The process of reduction which takes place in the mother-cells of ova and spermatozoa of animals is associated, as in plants, with far-reaching changes[1]; and the abundance of chromatin in these altered nuclei may, as also in plants, induce a rapid division into four of the nuclei. In plants this division into four takes place in the spore-mother-cells, without any direct relation to the sexual cells. That the constitution of the mother-nuclei induces the two divisions which rapidly follow each other, is shown by the fact that the mother-cells of the ova in animals give rise, on division, to products of unequal size: hence the subsequent division into four cannot be attributed to the form of the mother-cell. The division into four of the so-called paranuclei of the Infusoria doubtless takes place in relation with a corresponding reducing process[2], although under conditions which differ from those obtaining in the conjugating Infusoria. The uniformity in the phenomena of reduction and in the processes

[1] Compare O. Hertwig, Lehrbuch der Entwicklungsgeschichte, 4. Aufl., 1893, p. 32.

[2] Compare O. Hertwig, Die Zelle und das Gewebe, 1893, p. 216, where the literature is cited.

of division exhibited by the mother-cells of the ova and spermatozoa of those animals in which they have been minutely investigated, doubtless depends upon homology: the divisions into four after the process of reduction must have a common cause. The analogy is very striking between the much-discussed processes of splitting of the chromosomes in the mother-nuclei of animals, and the processes described by Farmer as taking place in the spore-mother-cells of Liverworts: for in *Pallavicinia* and in *Aneura* the number of chromosomes requisite for the two following divisions is provided at one and the same time in the nucleus of the spore-mother-cell. In these Liverworts, indeed, the abbreviation is the more marked, since a quadripolar nuclear spindle is formed, and the products of the longitudinal splitting of the chromosomes are simultaneously distributed to the four daughter-nuclei. It is of special purpose that I cite this particular case, for it alone, in plants, corresponds to the phenomena observed in animal ova: in these plants, as in animals, the double division follows on the process of reduction, and introduces the diminished number of chromosomes characteristic of the sexual cells. It must, however, be pointed out that the nuclear divisions with multipolar spindles to be found in the developing endosperm[1] of Angiosperms and in pathogenic tissues of animals, are not comparable with those here alluded to. For in those cases the division is not preceded by any internal changes in the nucleus; the number of the poles is variable, even accidental; and the number of the chromosomes distributed to the daughter-nuclei is not always the same.

I have already suggested that, in cases in which the product of the sexual act directly gives rise to the original generation, a process of reduction taking place at the commencement of germination is to be anticipated. It is possible to interpret in this sense the observations of Klebahn and Chmielewsky on the process of the germinating zygotes of

[1] Zellbildung und Zelltheilung, 3. Aufl., 1880, p. 18.

certain conjugate Algae. Klebahn [1] found that, in the germinating zygotes of *Closterium* and *Cosmarium*, the nucleus divides twice in rapid succession ; but the zygote then divides into only two cells, and only one nucleus for each of these two persists. Similarly, according to Chmielewsky [2], the nucleus of the zygote of *Spirogyra* divides into four, but the unicellular embryo subsequently possesses but a single nucleus. Klebahn has already suggested that, in these cases, a reduction in the number of the elements, probably by fusion with one another, must necessarily take place [3]: and Chmielewsky compares the phenomena in *Spirogyra* with the formation of polar bodies, a comparison to which Klebahn offers some objections [4], since the assumption of the formation of polar bodies after a sexual act would involve a modification of prevalent views as to the nature of these structures. Oscar Hertwig [5] considers 'that the processes described by Klebahn in the zygotes of Desmids, have the same object as the reducing divisions during the maturation of ova and spermatozoa.' 'As in that case the double division of the nucleus involves a reduction by half of the normal nuclear substance, before fertilization, and thus prevents an accumulation of nuclear substance which would otherwise take place as a consequence of the fusion of two nuclei in the sexual act ; so, in the Desmids, it would appear as if it were only after conjugation that a reduction of the nuclear substance is eventually effected, and thus the doubled mass of the nucleus in consequence of the fusion of two complete nuclei were brought back to the normal. The nucleus of the zygote, instead of dividing once, is divided into four by two immediately successive divisions ;

[1] Studien üb. Zygoten: I, Die Keimung von *Closterium* und *Cosmarium*, Jahrb. f. wiss. Bot., XXII, p. 415, 1891.

[2] The paper is in Russian, but a full abstract of it is given in Famintzin's Uebersicht der Leistungen auf dem Gebiete der Botanik in Russland während des Jahres 1890, p. 16.

[3] Loc. cit., p. 441.

[4] Studien üb. Zygoten : II, Die Befruchtung von *Oedogonium Boscii*, Jahrb. f. wiss. Bot., XXIV, p. 257. 1892.

[5] Die Zelle und das Gewebe, 1893, p. 225.

but the cytoplasm divides but once, and each half receives only one functional nucleus, whilst the remaining two nuclei, being superfluous, become disorganized.' O. Hertwig thus compares the processes occurring in these zygotes with the reducing divisions in the mother-cells of the ova and spermatozoa of animals which he defines as follows[1]: 'The essential feature of these divisions is that two closely related divisions follow each other in immediate succession, such that the second succeeds the first without an intervening resting-stage of the nucleus. Consequently the groups of nuclear segments resulting from the first division are immediately divided, without previous longitudinal splitting, into two daughter-groups. At the close of the second division the ripe ovum or spermatozoon receives only half so many nuclear segments, and, consequently, only half so much nuclein, as do the nuclei formed in an ordinary division in the same animal.' O. Hertwig thinks[2] that accurate counting of the nuclear segments in the various stages of division, would confirm his view that a reducing division takes place in the zygotes of the Desmids. If, however, my interpretation of the process is correct, then the reduction in the number of the chromosomes must take place at the commencement of the prophase of the nucleus of the zygote, and, as in so many other cases, its rapid division into four would be the consequence of changes undergone during the process of reduction.

When the morphological value of the polar bodies of the ova of animals is correctly estimated, and when the comparisons between the generative processes of animals and those of plants are accurately drawn, it becomes at once apparent how little justification there was for the attempt to find, in connexion with the ova of plants, structures which should correspond to the polar bodies of animals ; and how erroneous it was to regard the ventral canal-cells of Vascular Cryptogams and Gymnosperms as structures of this nature. This con-

[1] Loc. cit., p. 189 : see also Vergleich der Ei- und Samenbildung bei Nematoden, p. 65 of the separate copy.

[2] Loc. cit., p. 225.

sideration shows yet once more to how great an extent the advance of theoretical comprehension affects the correct apprehension of the problem to be solved, and how this correct apprehension may save a great deal of superfluous labour.

The reduction in number of the chromosomes takes place, among the higher plants, in the mother-cells of the spores, and it is consequently these which must be regarded as the first term of the new generation. They assert this their true significance in that they usually isolate themselves from cohesion with other cells and become independent, although this independence is only of practical utility in the case of the products of their division, that is, of the spores. Hence the centre of gravity of the developmental processes which take place in both micro- and macro-sporangia of Cryptogams and Phanerogams, does not lie in those cells, cell-rows, or cell-aggregates, which give rise to the sporogenous tissue and have been designated 'archesporium' by Goebel[1]. The archesporium still belongs to the sexually-developed asexual generation; it is only the spore-mother-cells which initiate the new sexual generation: consequently the presence or absence of a well-defined archesporium is not a matter to which importance should be attached. For the archesporium is merely the merismatic tissue from which the spore-mother-cells are derived, a tissue which is frequently, but by no means necessarily, differentiated from the surrounding tissues at an early stage; so that its differentiation cannot be of fundamental importance.

[1] Vergl. Entwicklungsgeschichte der Pflanzenorgane, 1883, p. 284.