THE BEHAVIOR OF THE CHROMOSOMES IN FERTILIZATION

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The problems of heredity are intimately related to the two critical stages in the life cycle of the higher organisms, the reduction divisions of sporogenesis in plants and of gametogenesis in animals, and the cytological processes of fertilization. The first of these has received much attention and is generally considered to be closely correlated with the segregation of the factors which determine the characters of the next generation. The union of the gametes at the time of fertilization is equally important, although we know little of the behavior of the chromosomes at this period. Recent research has made it desirable from both a cytological and a genetical standpoint to examine critically their behavior in the higher plants.

The behavior of the chromosomes in animals at the time of fertilization is comparatively well known. In some cases the sexual nuclei unite while in the resting condition so that the male and female contribution of chromosomes can not be distinguished. It has been shown, however, that in many species the male and female chromosomes are formed before the fusion of the sexual nuclei and that they may maintain the two distinct groups during the first division of the zygote. In some species the independence of male and female chromosomes has been traced through several divisions and even to later cleavage stages. Indeed there are reasons for believing that the male and female chromosomes may maintain their independence until gametogenesis. In all cases the first division of the fertilized egg appears to be essentially similar to any other normal somatic division.

In plants there is a great deal of variation in the behavior of the sexual nuclei in fertilization. In the algae it has been shown that in some cases the first division of the zygote is a reduction division. In *Spirogyra* (KARSTEN 1908) it has been found that the first division of the zygospore reduces the chromosome number from twenty-eight to fourteen.

ALLEN (1905) has found that the first division of the oospore nucleus of *Coleochaete* is also a reduction division. In all cases described the gametes unite completely and male and female chromosomes are not found in separate groups.

The fungi afford an interesting variety of phenomena in connection with fertilization. As HARPER (1910) points out, they have served to enlarge our conception of the sexual process elsewhere. In the rusts a fusion of two cells may occur independently of nuclear fusion as was shown by the work of BLACKMAN (1904) and CHRISTMAN (1905). The nuclei remain separate throughout the sporophytic phase until they fuse in the teleutospore just before reduction. The long period in which the nuclei exist side by side in the sporophyte without fusing and their final fusion prior to reduction is significant. There is no question but that the male and female chromosomes are independent in the sporophytic generation. No less striking are the conditions found in the Ascomycetes. HARPER'S (1905) work on the mildews has shown the existence of two nuclear fusions in the life cycle, one at the origin of the ascocarp where there is a fusion of differentiated gametes, and the other in the ascus. The nuclear fusion in the ascus is followed by three successive divisions which are thought to be correlated with the occurrence of the double fusion.

A most remarkable fusion which takes the place of the normal fertilization has been described in *Aspidium falcatum*, an apogamous fern, by Miss ALLEN (1914). In this case the sporophyte develops through a vegetative bud from the prothallus and has presumably the 1x number of chromosomes. Sixteen spore mother cells are present as in other ferns but these fuse in pairs to give eight cells from which a maximum of thirty-two spores may be developed. This fusion sometimes occurs while the spore mother cells are in the spireme stage so that two complete spiremes are present in the fusion nucleus. The fusion is followed immediately by the reduction division characteristic of spore formation.

The behavior of the chromosomes in fertilization has been described for many species of Gymnosperms. It was found by BLACKMAN (1898), FERGUSON (1904), and CHAMBERLAIN (1899), that in *Pinus* the male and female chromosomes are formed independently for the first division of the fertilized egg. The independent formation of male and female chromosomes has also been described in *Tsuga* (MURRILL 1900), *Juniperus* (NORÉN 1907), and *Abies* (HUTCHINSON 1915). All of these accounts are in general accord with the conditions found in some other plants and in many animals.

A most unusual behavior of the chromosomes in fertilization has been described in *Abies balsamea* by HUTCHINSON (1915). The chromosomes of the male and female gametes are formed independently as is the case in most conifers. As the two groups unite the chromosomes are said to become paired side by side and to twist about one another in a manner similar to their behavior in the reduction divisions. The number of chromosome pairs is haploid. According to HUTCHINSON there is then a *transverse* segmentation of the chromosome pairs. The resulting pairs of chromosomes are diploid in number, and of about half the length of the pairs before segmentation. The chromosomes are described as then separating to form $4x$ chromosomes, half of the number passing to each pole in the first division. The description is not supported by convincing figures.

CHAMBERLAIN (1916) in *Stangeria paradoxa* finds a similar pairing of chromosomes at fertilization. Although he apparently upholds HUTCHINSON'S conclusion he does not state that in this form there is a transverse segmentation of the chromosome pairs in the first division of the egg.

In the angiosperms there is no detailed account of the behavior of the chromosomes during the first division of the fertilized egg. In most cases the sexual nuclei fuse while in the resting condition. Cases have been described in *Lilium* (GUIGNARD 1891), *Cypripedium* (PACE 1907), and *Fritillaria* (SAX 1916), where the gamete nuclei were rarely in the spireme stage before fusion. The first division of the fertilized egg in angiosperms has been described by GUIGNARD (1891), ERNST (1902), GOLDSCHMIDT (1916), RENNER (1914) and the writer (SAX 1916), but in no case have the descriptions been complete and rarely has more than a single division figure been shown in any of the papers.

ATKINSON (1917) in a recent genetical paper on *Oenothera* has presented some results which he maintains can be explained only on the assumption that there is a segregation of factors in the F_1 zygote. He also states (p. 257):

"The germ plasm is peculiarly sensitive to shock from the meeting of sperm and egg, particularly when there is a genotypic difference between the two germ plasms. This results more or less in interchange, crossing over, dominance, as well as blending, of factors in the zygote, often accompanied by selection of factors into different associations in different zygotes giving rise to more than one hybrid type in the F_1 generation of crosses."

ATKINSON'S statements are largely theoretical, and, as DAVIS (1917)

has indicated, have little experimental and no cytological basis, yet we must consider such possibilities in the present study.

The purpose of this study is to consider not only the behavior of the chromosomes of the gametic nuclei, but also in the "triple fusion." Although it is questionable if we may consider the triple fusion as a real fertilization, yet so far as the inheritance of endosperm characters is involved it is quite comparable to the union of the gametic nuclei. For this reason and also because of the great importance of endosperm characters in cereal breeding, we will give some attention to the chromosomes in the triple fusion in both *Fritillaria* and *Triticum*. It is also necessary to describe briefly the development of the embryo sac in *Fritillaria* in order to understand the chromosome number found in the first division of the endosperm nucleus.

MATERIAL AND METHODS

The material for this paper was secured from about four thousand cases of fertilization in *Fritillaria pudica* Spreng. and not less than two hundred cases of fertilization in *Triticum durum hordeiforme* Hort. var. Kubanka. Flemming's stronger solution and chrom-acetic acid were used as fixatives for the *Fritillaria* ovaries. At the suggestion of Dr. OSTERHOUT about 0.5 percent of urea salts were added to the above fixatives and gave excellent results with the wheat ovaries. Besides assisting as a fixative, the urea salts reduce the surface tension of the solution and aid in the penetration of the fixative. Sections were cut from 10 μ to 20 μ thick. Modifications of Flemming's triple stain, and Heidenhain's iron alum haematoxylin were used in staining with good results.

THE CHROMOSOMES IN *FRITILLARIA*

A brief consideration of the chromosome number in the development of the embryo sac in *Fritillaria* is necessary before describing the later stages. Numerous counts of the chromosome number in the various stages of embryo sac development were made. In the heterotypic division of the megaspore mother cell twelve chromosomes pass to each pole. The second division usually appears to be normal and the four resulting nuclei each receive twelve chromosomes. These nuclei pass into the resting stage. The nuclei in the third division present considerable variation in respect to chromosome number. The two nuclei at the micropylar end of the embryo sac have the usual 1x number of chromosomes and when they divide twelve chromosomes pass to each pole. One of these resulting nuclei becomes the upper polar nucleus. The other

three constitute the nuclei of the cells of the egg apparatus, one of which functions as the egg. One of the two nuclei nearest the chalazal end of the sac in the four-nucleate stage disintegrates. The other, which has received twelve chromosomes from the previous division, has at the time of division not twelve chromosomes, but about twenty-four, and in the division about twenty-four chromosomes pass to each pole. One of the resulting nuclei becomes the lower polar nucleus. As a result of this development of the embryo sac the egg and upper polar nuclei each contain $1x$ or twelve chromosomes while the lower polar nucleus possesses approximately $2x$ chromosomes. The lower polar nucleus and the normal antipodal nucleus are considerably larger than the other nuclei of the embryo sac, presumably because of the $2x$ number of chromosomes which they contain.

The general behavior of the sexual nuclei in *Fritillaria* has been described in an earlier paper (SAX 1916). The contents of the male nuclei while free in the embryo sac were found to be in an irregular dark-staining network. The male nucleus soon after coming in contact with the egg nucleus loses this netlike structure of its chromatin and passes into the usual resting condition. The union of the sexual nuclei occurs while they are in the resting condition as shown in figure 1, plate 1. The male nucleus can be recognized by its slightly smaller size. In both nuclei there are nucleoli, and the chromatin is in a reticular network. In the cases figured the nuclei are flattened at the point of contact but in some cases the egg nucleus is somewhat indented. The cytoplasm is distributed uniformly throughout the lower part of the egg cell, while the upper part is almost entirely occupied by a large vacuole. The gametic nuclei remain distinct for a comparatively long time, often until the endosperm has reached the four- or eight-nucleate stage.

The disappearance of the adjacent nuclear membranes of the sexual nuclei results in a fusion nucleus which is also in the resting stage. At first there is a trace of the outlines of the two nuclei but later the fusion nucleus shows no indication of its binucleate origin. At this stage all of the chromatin stains alike and it is impossible to distinguish the male and female chromatin as separate contributions. Such a case is shown in figure 2. The chromatin is in the resting condition and more nucleoli are present than in the nucleus of the unfertilized egg.

The fusion nucleus remains in the resting stage but a short time. The chromatin forms a fine threadlike structure and the nucleoli begin to disappear. Figure 3 shows an early spireme stage where the spireme is not completely formed. Delicate threads can be seen among the

heavier chromatin threads. The spireme thread thickens until it appears as illustrated in figure 4. Here the nucleoli have not entirely disappeared and traces of the nuclear membrane may be seen. The broken appearance of the spireme is partly due to the passage of the microtome knife through the edge of the nucleus. But in many other spiremes examined it was also impossible to demonstrate the presence of a single continuous spireme.

After the formation of the spireme the nuclear membrane disappears entirely. The spireme contracts and becomes somewhat thicker just before the segmentation into chromosomes occurs. The segmentation of the spireme is shown in figure 5. The nuclear membrane has completely disappeared and the segmenting spireme has contracted. Surrounding the nuclear cavity is a dense area of cytoplasm from which delicate threads pass to the newly formed chromosomes.

A significant variation in spireme formation of the zygote has been described for *Fritillaria* in an earlier paper (SAX 1916, fig. 21). In this case the sexual nuclei were found in the spireme stage before fusion. This condition is very rare in the fusion of the egg and male nuclei, but is not uncommon in the nuclei of the triple fusion.

The chromosomes in the metaphase of the first division are long and often cover much of the spindle. The spindle is bipolar and the chromosomes are in a single group and not paired. The number of chromosomes at this time is twenty-four. A longitudinal section of the metaphase of the zygote is presented in figure 6. All of the chromosomes are not shown in this figure, but in this and the two following figures the proportion of chromosomes omitted, due to their presence in an adjacent section, is about the same. The cytoplasm at this time is more uniform than in the preceding stages.

As the chromosomes divide twenty-four pass to each pole. There is no evidence that the male and female chromosomes are separated in two groups. The spindle is typically bipolar. The anaphase of the first division of the zygote is shown in figures 7 and 8. In figure 7 the chromosomes are not so large as in the metaphase. In figure 8 the chromosomes are nearer the poles and they are larger than in the preceding figure. The spindle at the lower end appears to be three-parted, possibly due to the fact that it is cut somewhat obliquely. The other pole has no appearance of being divided. In neither case figured nor in many others examined is there any evidence that the chromosomes pair.

In the telophase the chromosomes form a single compact group at

either pole. The fibers between the poles thicken and the cell plate is formed. A clear telophase is shown in figure 9. Although the chromatin groups are very compact the outlines of the chromosomes are still apparent.

The second division of the embryo appears to be the same as any other normal somatic division. Often one of the cells of the two-celled embryo divides before the other, but usually they divide at the same time.

The second male nucleus and the two polar nuclei usually fuse in the resting condition. Often, however, the nuclei are found in the spireme stage before they fuse and occasionally before the upper polar nucleus and male nucleus have joined the lower polar nucleus. A striking case of the latter condition is shown in figure 10. The nuclei are in early spireme stages. The difference in the size of the nuclei is evident. In this triple fusion the large lower polar nucleus contributes about $2x$ chromosomes while the upper polar nucleus and the male nucleus each contribute $1x$ chromosomes. Thus $3x$ chromosomes are contributed by the maternal parent and only $1x$ by the male parent.

The number of chromosomes in the metaphase of the first division of the "endosperm nucleus" is approximately $4x$ or forty-eight. From figure 11 it is evident that this division appears to be normal. There is no segregation of chromosomes into groups, no pairing of chromosomes, and the spindle is not multipolar.

The chromosomes of the metaphase split longitudinally and about forty-eight daughter chromosomes pass to each pole. In the anaphase shown in figure 12 it was impossible to show all of the chromosomes. Apparently an equal number of chromosomes pass to each pole and the division in every way appears to be regular.

THE CHROMOSOMES IN TRITICUM

The mature embryo sac in wheat consists of the egg cell, two synergids, two polar nuclei in the primordial endosperm, and a large number of antipodal cells. The cytoplasm of the egg cell is more or less vacuolate near the periphery, but a denser layer is found around the nucleus. The egg nucleus contains one large nucleolus and often several small ones. The chromatin is arranged in a loose irregular network. The polar nuclei come in contact with each other, but do not fuse, before fertilization. They are very large and are about equal in size. The cytoplasm of the "Endospermanlage" extends from the polar nuclei to the egg apparatus.

The male nucleus as it enters the egg cell is small, elongated and its

contents are so dense that it appears almost homogeneous in structure. It takes a brilliant red stain with the safranin and is easily recognized. A male nucleus free in the cytoplasm of the egg cell is shown in figure 13. It is long and curved at the ends. The structure of the male nucleus appears almost homogeneous except for small vacuoles and darker-staining particles. There is no trace of a separate cytoplasmic sheath around the male nucleus.

The male nucleus when in contact with the egg nucleus is often more or less coiled, but has the same structure as when free in the egg cell. Such a case is presented in figure 14. Immediately around the male nucleus the cytoplasm is less dense and a few delicate threads cross this vacuolate area. This condition was also found in other preparations. The chromatin of the egg nucleus at this time is still in the resting condition.

The male nucleus apparently penetrates the egg nucleus before increasing in size. Many stages were found where the compact spireme of the male nucleus could be seen inside of the membrane of the egg nucleus. Its appearance is striking and unmistakable because of its density and darker-staining properties. In figure 15 the male nucleus is shown inside of the egg nucleus near the membrane. It has increased in size and the irregular compact spireme thread can be seen. There are several small nucleoli in the male contribution of chromatin. The chromatin of the egg nucleus is in the early spireme stage and portions of the spireme thread are often more or less parallel. In figure 16 the early spireme of the male chromatin is especially distinct. Its dark-staining closely coiled spireme is quite distinct from the loose spireme of the egg nucleus. The nuclear membrane is still apparent at this stage. In both cases figured the large nucleolus of the egg nucleus is shown quite separate from the male chromatin.

The segmentation of the two spiremes of the zygote results in $2x$ chromosomes which are unpaired. The metaphase of the first division of the fertilized egg is illustrated in figure 17. There are approximately twenty-eight chromosomes to be seen in this section. Several pieces of chromosomes, or possibly whole chromosomes, were found in an adjacent section. There is no indication that the chromosomes contributed by the male and female gametes lie in separate groups and they are not in pairs. In several chromosomes shown in this figure a longitudinal division may be seen. This longitudinal splitting of the chromosomes continues until all of the chromosomes have divided, as illustrated in figure 18. There are about twenty-one pairs shown in this figure and

there are six or seven pairs in an adjacent section. These split chromosomes are comparatively long and are often curved. The daughter halves are most widely separated at the center and in some cases they appear to be slightly twisted about one another. After the completion of the longitudinal fission $2x$ daughter chromosomes pass to each pole. An anaphase of the first division is shown in figure 19. The daughter chromosomes are about as long as the mother chromosomes of the metaphase and are often curved. The spindle is clearly bipolar. The dense layer of cytoplasm so conspicuous about the egg nucleus in the earlier stages is apparent around the spindle.

The chromosomes pass to each pole in a single group. In figure 20 approximately twenty-eight chromosomes may be counted at each pole in the late anaphase. The chromosomes have shortened a little and are in compact groups. It will be noted that the dense area of cytoplasm in the egg cell is for the most part around only the upper pole, while the chromosomes of the lower pole lie in the more vacuolate cytoplasm. The chromatin of the telophase passes into the resting stage, a cell plate is laid down and the two-celled embryo is formed.

The male nucleus which unites with the polar nuclei appears to be quite similar to the one which unites with the egg nucleus. In figure 21 the triple fusion is shown. The dark-staining male nucleus is coiled at the side of one of the polar nuclei. The polar nuclei are large and of about equal size. Each contains a large nucleolus and the chromatin is in granules in an open network.

At the time of spireme formation the chromatin contributed by each of the three nuclei may be distinguished. Such a case is shown in figure 22. Here the spireme of the male nucleus is in a comparatively compact ball at the upper side of the figure. The spireme of each polar nucleus is rather open, very irregular, and quite distinct. The nucleolus and outlines of each polar nucleus may be seen, but the nuclear membranes have almost entirely disappeared.

The chromatin of the male nucleus and polar nuclei maintain their independence not only in the spireme stage, but apparently also in the early division stages. Figures 23 A and 23 B are drawn from adjacent sections of the first division of the triple fusion nucleus. This is the early metaphase when the chromosomes are on the nuclear plate. There are approximately forty chromosomes to be counted. The chromosomes appear to be in three groups, each group containing approximately an equal number of chromosomes. The spindle in figure 23 A is clearly two-parted at the upper end and more or less so at the lower end. The

spindle in figure 23 B appears to be distinct from the spindles in figure 23 A. Later stages in the first division of the triple fusion nucleus were not observed, but judging from many counts of later endosperm divisions, the first division is regular and an equal number of chromosomes pass to each pole. The fact that there are about forty chromosomes in the endosperm divisions indicates that the polar nuclei each contribute $1x$ chromosomes. After the first division the endosperm nuclei divide rapidly and cell walls are not formed in the early development of the endosperm.

DISCUSSION

In many animals and in most gymnosperms the independence of the formation of the male and female chromosomes following fertilization is unquestioned. There are a few cases in the angiosperms where the independent formation in the zygote of the spiremes from the gametes has been demonstrated. In an earlier paper (SAX 1916) the writer described the gamete nuclei of *Fritillaria*, in rare cases, as in the spireme stage before fusion. In wheat the spireme of the male and female nuclei are formed separately even though the male nucleus is within the egg nucleus at the time of spireme formation (figures 15 and 16). In both *Fritillaria* and *Triticum* the nuclei of the triple fusion may be in the spireme stage before fusion. In *Triticum* the chromosomes contributed by each nucleus appear to be in more or less separate groups even in the metaphase of the first division. Although there is considerable variation in the condition of the sexual nuclei at the time of fusion, I believe that we are justified in assuming that the male and female chromosomes are formed independently in the zygote, even in cases where the nuclei fuse in the resting condition.

The behavior of the chromosomes during the first division of the egg in *Fritillaria* and *Triticum* is essentially not different from any other normal somatic division. There is no indication that the chromosomes of the male and female gametes are in separate groups, or that they pair in the first division of the zygote. In *Triticum* the chromosomes in the metaphase, when first split longitudinally, appear much like certain stages in *Abies*, which HUTCHINSON (1915) interprets as a pairing of chromosomes. But in *Triticum* the chromosomes of the metaphase before splitting are distinctly $2x$ in number and after splitting there are $2x$ pairs. If a pairing of male and female chromosomes occurred we would expect to find only $1x$ pairs of chromosomes.

In neither *Triticum* nor *Fritillaria* is there anything comparable to

a reduction division in the fertilized egg nor are there any irregularities which might account for a zygotic segregation of factors. It is possible, as ATKINSON (1917) suggests, that there is a "shock" as the result of the meeting of genotypically different germ plasms, which may cause an irregular behavior of the first division of the zygote. The only cytological work which might support this theory is that of GOLDSCHMIDT (1916). He explains the occurrence of patrocliny in *Oenothera* crosses as a result of merogony. There is no good cytological evidence of any behavior which would explain a segregation of factors during the first division of the zygote. The segregation of factors in the F_1 as described by ATKINSON, might be accounted for on the assumption that in *Oenothera* the chromosomes of the zygote behave as described in *Abies*. But this assumption would lead into other problems more difficult to explain. It is much more probable, as DAVIS (1917) has suggested, that ATKINSON is dealing with genotypically impure parent species, and that his assumptions concerning the segregation of factors in the zygote are entirely unnecessary.

The behavior of the chromosomes of the zygote in *Abies*, as described by HUTCHINSON (1915), if verified, would be of considerable genetical significance to those who consider the chromosomes the bearers of the hereditary factors. According to HUTCHINSON the chromosomes of the male and female nuclei pair in the zygote so that there are $1x$ pairs. Each pair segments transversely, forming $2x$ pairs of chromosomes. The chromosome segments separate and $2x$ chromosomes pass to each pole. There is no longitudinal division of the chromosomes as is the case in the usual type of somatic division. If we assume that the hereditary factors are located in the chromosomes it is obvious that if homologous chromosome segments pass to opposite poles the hereditary factors will be segregated according to the usual Mendelian behavior. We would then expect a segregation of characters in F_1 individuals in crosses when the parents differ in Mendelizing characters. If we assume that not only are the hereditary factors located in the chromosomes, but also in a definite linear arrangement in the chromosomes, it is evident that the chromosomes can not divide longitudinally at one time and transversely at another time, as described in *Abies*, without causing chaos in the distribution of the hereditary factors. Furthermore if this process continued through several generations each chromosome would finally contain but a single factor and most of the factors would be lost. Certainly the behavior of the chromosomes in *Abies*, as

described by HUTCHINSON, can not be brought into harmony with the theory of a linear arrangement of the factors in the chromosomes. To be sure the latter theory is only a working hypothesis, but the research of MORGAN and his students, and other experimental work, can best be explained by it.

If in *Abies* there is a double "reduction," one at the time of sporogenesis, the other during the first division of the zygote, we might expect a compensating fusion at some period in the life cycle. A comparable case is found in the mildews where the double fusion is thought to be followed by a double reduction in the ascus. In view of the present study and the theoretical questions raised by HUTCHINSON'S results, it would be well to examine more critically the behavior of the chromosomes in the first division of the zygote in *Abies*.

The pairing of the chromosomes in somatic cells has been described in *Galtonia*, *Funkia*, *Oenothera*, *Thalictrum* and *Yucca*. It appears that the pairing of the chromosomes may take place at the time of fertilization, as HUTCHINSON maintains is the case in *Abies* and as CHAMBERLAIN (1916) has described in *Stangeria*, or that the pairing may not occur until synapsis as is true in the rusts. There may be intermediate examples where the chromosomes pair at various times in the sporophytic life cycle. It is possible as Miss FRASER (1912) suggests, that "the clearest cases of Mendelian inheritance will perhaps be those correlated with a late association of the chromosomes in pairs." Experimental work, however, makes it appear probable that the chromosomes are quite independent, and that if there is any influence or interchange of factors between chromosomes it does not occur until synapsis. According to HARPER (1910) the behavior of the chromosomes in the rusts "is certainly strongly suggestive that synapsis and its accompanying phases represent a stage of mutual influence if not of interchange of physical material between the chromosomes much more intimate than any which has preceded it in the life of the sporophyte."

In *Fritillaria* and in *Triticum* there is no evidence that the chromosomes pair in fertilization. The male and female spiremes are formed independently. At synapsis there is a pairing of male and female chromosomes. We might expect that here two spiremes are formed, one male and one female, as in fertilization, and that these spiremes or portions of these spiremes, pair side by side (parasynapsis), rather than expect that homologous chromosomes become arranged alternately end to end and then pair as in telosynapsis. In *Aspidium* where the

spore mother cells fuse while in the spireme stage there is a reduction division immediately following. In this case there is probably a pairing of homologous chromosomes as is generally thought to occur in reduction divisions. It is more difficult to assume that here the chromosomes should follow the telosynaptic method of reduction than to assume that the spiremes conjugate side by side as in parasynapsis. In either case, however, the physical difficulties seem to be very great. If there is a somatic pairing of chromosomes in cases where the telosynaptic method of reduction occurs we might expect the male and female chromosomes to become arranged alternately end to end at or soon after fertilization.

In the development of the embryo sac in *Fritillaria* twenty-four chromosomes are found in the metaphase of the third division at the chalazal end of the embryo sac, and as a result of this division the lower polar nucleus receives about $2x$ or twenty-four chromosomes. A similar doubling of the chromosome number at the chalazal end of the embryo sac has been described in *Lilium martagon* by GUIGNARD (1891), MOTTIER (1897), SARGANT (1896), and STRASBURGER (1908). According to STRASBURGER this doubling is due to a premature longitudinal splitting of the chromosomes and is dependent on the food supply of the plant. In *Fritillaria* the lower polar nucleus which contains about $2x$ chromosomes is considerably larger than the upper polar nucleus which contains $1x$ chromosomes. Since both nuclei are surrounded by a common cytoplasm, the difference in size is probably due to the difference in chromosome number. In *Triticum* the polar nuclei are of equal size and so probably have the same chromosome number, presumably $1x$ each. The difference in size between the polar nuclei and the egg nucleus is apparently due to the nuclear cytoplasmic relations.

In the triple fusion in *Fritillaria* there are about $3x$ chromosomes contributed by the female parent and $1x$ chromosomes contributed by the male parent. If we assume that the hereditary factors are borne by the chromosomes, then the female parent contributes three sets of factors and the male parent contributes only one set. This phenomenon is of considerable importance in relation to the multiple factor hypothesis. For instance HAYES and EAST (1915) have found in a particular cross in corn that the endosperm character borne by the mother was always dominant. One of the parents used had a starchy endosperm, the other a corneous endosperm. Either way the cross was made the F_1 endosperm was always of the same kind as the endosperm of the mother parent. This type of inheritance is explained on the assumption that the

two factors contributed by the mother, as a result of polar fusion, are always dominant over the single factor contributed by the male parent. The effect of multiple factors is clearly demonstrated here, and if the female parent contributes not two, but three factors, the maternal contribution should be still more potent.

It is unnecessary to consider the morphological character of the endosperm because this subject has been dealt with fully by EAST (1913). It may be added, however, that the development of the endosperm is independent of the development of the embryo. While working with Mr. E. F. GAINES at WASHINGTON STATE COLLEGE, I found about a dozen cases in wheat where the endosperm had developed normally and yet no embryo was present. One such case has been found in corn. Such a condition, although very rare, is of theoretical interest.

The significance of double fertilization has been a puzzle to many botanists. The suggestion of NEMEC (1910) seem to be the best explanation and has some experimental proof. NEMEC maintains that an endosperm whose characters are contributed by both parents is better suited to the nourishment of the embryo than an endosperm of wholly maternal origin. STINGL'S (1907) work supports this theory. He has transferred embryos of various cereals to their own and other endosperms. In most cases the embryo grew better on its own endosperm than on that of another genus. Preliminary experiments of my own in transferring hybrid corn embryos to the parent endosperms and *vice versa*, have not as yet given definite results. In view of STINGL'S results and my own work it appears that the experimental error is too large to permit small differences in adaptability of embryo and endosperm to be noted. If we assume that a hybrid endosperm is better able to nourish the hybrid embryo than an endosperm derived from the mother only, we might expect that "wider" crosses could be made among plants where the endosperm is derived from both parents.

CONCLUSIONS

A complete series of stages in the first division of the fertilized egg has been found in both *Fritillaria pudica* Spreng. and in *Triticum durum hordeiforme* Host. var. Kubanka.

In *Fritillaria* the male and female nuclei are of about equal size before fusion. The sexual nuclei usually unite while in the resting condition, although occasionally they are in the spireme stage at the time of fusion. The presence of a single continuous spireme in the zygote

could not be demonstrated at any time. In the first division of the fertilized egg each chromosome contributed by the male and female gametes splits longitudinally and twenty-four chromosomes pass to each pole.

In *Triticum* the male nucleus is small and almost homogeneous in structure even when in contact with the egg nucleus. The male nucleus enters the egg nucleus and forms a separate compact spireme. At the same time the spireme of the egg nucleus is formed. In the first division approximately twenty-eight chromosomes are found in the metaphase. These chromosomes split longitudinally and the $2x$ number pass to each pole.

In both *Fritillaria* and *Triticum* the first division of the zygote appears to be essentially like any other normal somatic mitosis. The male and female chromosomes are formed independently; they are not found in separate groups, nor do they pair. There is no chromosome behavior at this time which might account for a zygotic segregation of factors.

In the triple fusion in *Fritillaria* the nuclei are often found in the spireme stage before fusion. The first division of the endosperm nucleus appears to be very regular. The chromosomes are not paired and there is no segregation of the chromosomes contributed by each nucleus. In this division about $4x$ or forty-eight chromosomes pass to each pole. This number is due to the fact that there is a doubling of the chromosomes at the chalazal end of the embryo sac in the third division and as a result the lower polar nucleus receives about twenty-four chromosomes. Thus in the triple fusion the female parent contributes $3x$ chromosomes and the male parent contributes only $1x$ chromosomes. This phenomenon is of interest in relation to the multiple factor hypothesis.

In *Triticum* the nuclei involved in the triple fusion form separate spiremes. There is evidence that the chromosomes contributed by each nucleus may remain more or less separate even in the metaphase of the first division. Each nucleus contributes $1x$ or about fourteen chromosomes.

In both *Fritillaria* and *Triticum* the chromosomes of the triple fusion are formed independently, there is no pairing of chromosomes and the first and following divisions appear to be regular.

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EXPLANATION OF PLATES

All drawings were made with the aid of the camera lucida. The figures were drawn from single sections with the exception of figure 22. The magnification is as follows: Figure 3, 1120 diameters; figure 21, 900 diameters; all other figures, 1200 diameters. No reduction.

PLATE I.—FRITILLARIA

- FIGURE 1.—Male nucleus and egg nucleus in contact.
FIGURE 2.—Complete fusion of male and egg nuclei.
FIGURE 3.—Early spireme of fertilized egg.
FIGURE 4.—Spireme of fertilized egg.
FIGURE 5.—Segmentation of spireme. Formation of chromosomes.
FIGURE 6.—Metaphase of the first division of the fertilized egg.
FIGURES 7, 8.—Anaphase of the first division of the fertilized egg.
FIGURE 9.—Telophase of the first division of the fertilized egg.
FIGURE 10.—Early triple fusion.
FIGURE 11.—Metaphase of the first division of the endosperm nucleus.
FIGURE 12.—Anaphase of the first division of the endosperm nucleus.

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PLATE 2.—TRITICUM

- FIGURE 13.—Male nucleus in the egg cell.
FIGURE 14.—Male nucleus in contact with the egg nucleus.
FIGURES 15, 16.—Male nucleus within the egg nucleus.
FIGURE 17.—Metaphase of the first division of the fertilized egg.
FIGURE 18.—Late metaphase of the first division of the zygote.
FIGURE 19.—Early anaphase of the first division of the zygote.
FIGURE 20.—Late anaphase of the first division of the zygote.
FIGURE 21.—Early triple fusion.
FIGURE 22.—Spireme stage in the triple fusion.
FIGURES 23A, 23B.—Adjacent sections of the early metaphase of the first division of the triple fusion nucleus.

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