MATURATION IN DIPLOID AND TRIPLOID TOMATOES¹

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INTRODUCTION

In the past few years numerous observations have been made which seem to indicate that abnormalities in chromosome distribution are less uncommon than was formerly thought. Triploidy appears to be one of the more frequent types, having been described for mosses, Morus, Canna, Datura, Hyacinthus, Uvularia, Oenothera, and Drosophila. Up to the present time no complete study of the early meiotic prophase of a triploid has been published although several authors have reported on the chromosome distribution and a few have studied the forms of the trisomes at diakinesis. It was thought that such an investigation might be useful both as a means of helping in the clarification of the vexed question of parasynapsis *versus* telosynapsis, and of determining the extent to which crossing over may be expected between members of a trisomic set of chromosomes in the tomato.

MATERIAL AND METHOD

Roots, anthers and ovaries from a triploid plant of Solanum lycopersicum L. were fixed in chrom-acetic-urea and stained with Heidenhain's

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iron haematoxylin. Anthers were also fixed in Flemming's strong. A short description of the plant and its origin has already been published (LESLEY and MANN 1925). Chromosome counts from pollen mother cells were made from smears stained by Belling's aceto-carmine method. Sections of whole buds were made to determine the time of maturation in the ovules in relation to that in the pollen mother cells. It was found in general that ovaries contained the desired stages when the pollen mother cells were in cytokinesis or immediately thereafter. Sometimes different anthers of the same flower were in very different stages and many degenerating anthers were found in both diploid and triploid buds. The genetics of the triploid plant is being investigated by J. W. LESLEY and it is to him that I owe the privilege of working on this material.

MATURATION IN THE DIPLOID

Seriation of stages

The maturation processes in the tomato were first studied from megaspore mother cells but since ovarian material does not give any adequate clue to seriation of certain critical stages, longitudinal sections of anthers were used for comparison. As long ago as 1904 BERGHS stated that neighboring cells in the anther sac of *Lilium lancifolium* often show a graded series of stages which give one a clue to the natural seriation in meiosis. JANSSENS (1905) speaking of the natural seriation shown by the testis of Batrachoceps says (p. 380)

"We find ourselves then in the presence of an organ, which from the point of view of seriation of stages, offers the same advantage as the locules of the anthers of the Liliaceae."

No one anther provides a complete series in tomato, but a study of numerous anthers gives one an overlapping set of series from which most of the desired facts may be ascertained. The youngest stages are found at the proximal end; the oldest may be either at the distal end or near the center of the locule, usually the former. While the two locules of one anther contain approximately the same stages of meiosis, one may be slightly in advance of the other. The stages in the different anthers have been arranged in their natural order of progression from presynapsis to cytokinesis and are given below:

(1) Presynapsis (usually occupies whole locule)

- (2) Leptotene to synezesis
- (3) Synezesis to pachytene
- (4) Synezesis (few cells at base of anther) pachytene to first anaphase
- (5) Diakinesis to first telophase or sometimes second anaphase

- (6) First metaphase to second anaphase
- (7) Second anaphase to cytokinesis
- (8) Cytokinesis and young pollen cells

Nuclear changes during meiosis

In the tomato one finds a true spireme (figure 1) and typical parasynapsis (figure 2), the stages and their sequence conforming very accurately to those described by BERGHS (1904). These descriptions differ from the typical parasynaptic scheme as presented by SHARP (1921, p. 231, scheme A, figure 83) in that there is a continuous spireme and that following the pachynema stage (figure 3) the threads lose some of their affinity for stain and again appear as thin paired threads which then shorten, thicken, and become separated as the pairs of diakinesis (figures 4 to 8). One finds the usual rings, V's, and laterally paired rods at diakinesis, consisting of homologues variously attached to one another (figure 8). The changes observed in the tomato following pachynema are of the same type as those which occur in animal oögenesis, but they are less extreme. The same stages were found in both \circ and σ sporogenesis. No figures of synezesis have been presented, because, as usual, it is difficult to see much at its height. It was noted, however, that longitudinal pairing of threads precedes synezesis, that they remain paired during it, and emerge in the same condition. The chromosome number of the normal diploid tomato is N = 12, as WINKLER (1910) stated. Twelve pairs and regular meiotic divisions are certainly the usual thing. The chromosomes are short rods showing no distinct individuality.

Orientation of spindles in anther loculi

It was noted with great interest that the pollen mother cells are not oriented at random in the anther loculi at and immediately following the first meiotic division, but that a longitudinal section at right angles to the septum between loculi shows only lateral views of spindles. As one would expect, therefore, cross sections show many polar views of first-metaphase and anaphase groups. The second-metaphase figures are oriented at random.

The cytoplasm during meiosis

During meiosis the cytoplasm shows certain typical changes which are probably the result of the division phenomena. At and before diakinesis it appears to be of fairly uniform and densely granular structure. Just after the disappearance of the nuclear wall, however, one notes a denser area about the chromosome group at about the place which the nuclear GENETICS 11: My 1926

Legend for Plate 1

FIGURES 1 to 7.—Nuclear changes in successive stages in the prophases of meiosis in the embryo mother cell of the diploid tomato. FIGURE 8.—Typical diakinesis chromosomes in the diploid.



LESLEY, M. M., MATURATION IN DIPLOID AND TRIPLOID TOMATOES PLATE 1

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wall previously occupied, while the region between this and the chromosomes is practically free of granules and that between the granular area and the cell wall is slightly less opaque. WINKLER (1916) shows this cytoplasmic differentiation in his figures of first-metaphase plates. During the development of the first-metaphase plate and in early anaphase this sphere becomes unequal in thickness, usually appearing as a wedgeshaped mass, now very distinctly denser than the rest of the protoplasm and taking a blue stain in haematoxylin. In early telephase, just before and while the nuclei are enlarging, the cytoplasm again appears almost uniformly granular, although the region immediately surrounding the barrel-shaped remnant of the spindle is slightly denser than the rest of the cytoplasm. As the nuclei enlarge this denser region is compressed to form a spool-shaped body which stains blue, and after the nuclear walls disappear this remains, the rest of the cytoplasm being almost granulefree (clear white after fixation in chrom-acetic-urea). From this time until early second telophase this densely granular structure usually retains its form, but in a few cases when the two spindles lie in slightly different planes and at a slight angle to each other, it has been seen as two crescent-shaped halves each of which conforms to one side of a spindle. As the nuclei of the second telophase begin to form and the spindles are rotating to their final position before cytokinesis (such as to give the usual tetrahedral arrangement of pollen cells) the cytoplasm again becomes fairly uniform in density. The chief interest in the process described above lies in its bearing on the times and to some extent the method of cytoplasmic movements during the nuclear division. The spindle is obviously a very effective barrier to lateral transfer of cytoplasmic granules and when two spindles are present each forms a center of activity which affects the intervening region very slightly if at all. At the telophase, as the nuclei take in fluid, general cytoplasmic movements occur, resulting in an even distribution of granules. This is probably facilitated at the second division by the movement of the spindles. Somewhat similar changes in the cytoplasm are seen during mitrosis in root-tip cells.

Number of archesporial cells

In the diploid tomato one occasionally finds a several celled archesporium, and in a very few cases two megaspore mother cells were seen in meiosis. A partially sterile double trisomic plant (26 chromosomes) showed this condition very frequently, occasionally as many as three cells undergoing reduction in one ovule. Such twin and triplet megaspore GENETICS 11: My 1926

LEGEND FOR PLATE 2

FIGURES 9 to 12.—Nuclear changes in the prophase of meiosis in the triploid tomato, showing alternate double and single threads.

FIGURE 13.—A pre-diakinesis stage from a pollen mother cell of the triploid which shows the alternate single and double threads especially clearly. Note that the double portions of the trisomes are comparable to the paired chromosomes of the diploid (figure 7).

FIGURES 14a and 14b show most of the 12 trisomes in early diakinesis of an embryo-sac mother cell.

FIGURE 15 a to d shows four slightly different trisome formations from a pollen mother cell.

FIGURE 16.—A typical somatic metaphase of the triploid.



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mother cells may or may not mature synchronously. In one case one megaspore mother cell was in diakinesis, while its mate was in first metaphase. COULTER and CHAMBERLAIN (1912, p. 62) give evidence which shows that a many-celled archesporium is sometimes found in a number of the Sympetalae. The triploid, like the diploid, shows two megaspore mother cells only rarely. Two megaspore mother cells in synapsis are shown in text figure 1.



FIGURE 1.—Two embryo-sac mother cells undergoing reduction within the same megasporangium.

MATURATION IN THE TRIPLOID

Nuclear changes during meiosis and assortment of chromosomes

As in the diploid tomato the seriation seen in longitudinal sections of anthers was of great service. At the earliest stages the nucleus contains unpaired threads, most, at least, of which are united to form a spireme. One next finds a spireme which is double parasynaptically in some parts and single in others (plate 2, figures 9, 10, 11). As this shortens, the doubled regions of the spireme may remain in contact throughout their length, or they may only remain attached at the tips of the chromosomes (plate 2, figures 10, 11 and 12). In diakinesis trisomes most frequently consist of two chromosomes attached at both tips to form a ring, the third chromosome being united to them at one of these points of union (plate 2, figures 14a and 15a). They also appear as a pair laterally united GENETICS 11: My 1926 with the third element joined to the tip of one member of the pair (figures 14a and 14b), or as three chromosomes joined end to end (figures 15c and 15d). The latter type results from the opening out of the paired chromosomes to form a V (figure 15, b, c and d). It was noted above that the chromosomes of the diploid form rings, parallel rods, or V's consisting of homologues joined end to end so that the configurations seen in the triploid differ only in the presence of the unpaired element which bears a telosynaptic relation to the trisomic group. This third element often shortens more slowly than the pair and seems not to be as firmly attached to it as they are to each other, since a few univalents are not uncommonly seen at the late prophase.

When this study was begun it was thought that one could differentiate in a triploid between splitting and parasynaptic union of chromosome threads, because it seemed possible that the three threads might all unite parasynaptically. This does not occur, but the alternation of paired and unpaired threads in the spireme together with their relationship to the chromosomes in diakinesis is quite as convincing evidence that the two threads seen in the diploid are parasynaptically united and are not the result of longitudinal division of the spireme. It is also of interest that the third element is joined telosynaptically to the other two chromosomes. One usually finds 12 trisomes at diakinesis, but at a later stage, in the first metaphase, one to several single chromosomes are sometimes seen. At this time the trisomes look like three balls of the same size. The univalents are easily distinguished from the bivalents or trisomes. Four megaspores may be formed, or one of the first two cells may degenerate without further division. Apparently some embryo formation occurs in the triploid, but the material was not well enough fixed to show anything about the proportion of embryos that begin to develop. It is known (LESLEY and MANN 1925) that the triploid has some viable ovules, since a few seeds result from fertilization with diploid pollen. The pollen of the triploid has thus far proven useless either on diploid or triploid plants. From the cytological observations one would expect a high percentage of sterility, but a study of meiosis does not show why it should be as bad as the experimental results demonstrate it to be. The following chromosome counts taken from 94 second-metaphase figures from pollen mother cells show that the chromosomes assort at random:

Distributi	on of	pollen	moth	er cell	s wit	n res ₁	pect t	o nun	iver o	j cnro	moso	mes		
Number of chromoso	mes	12	13	14	15	16	17	18	19	20	21	22	23	24
Frequency			1	6	4	10	23	22	13	4	7	0	2	1

TABLE 1

Lagging chromosomes are sometimes seen, and these very occasionally divide or are left in the spindle where they form micronuclei, but the divisions are usually clean. Cytokinesis gives rise to four to eight pollen cells, usually four, which sometimes differ in size. Occasionally one pollen cell divides, forming a little tetrad.

A longitudinal section of an ovary of the triploid in which the megaspore mother cells were mostly in synapsis had a sac on one side of the style in which a mass of microspore mother cells were at the same stage, while the normal anthers of the same flower contained pollen mother cells in early cytokinesis.

LITERATURE AND DISCUSSION

The term triploid has been used rather loosely to designate plants which have originated by hybridization between species, one of which has twice the chromosome number of the other, as well as for forms in which the triploid number of chromosomes has resulted from the doubling of one set of chromosomes within a species. This fact should be kept in mind in any discussion of triploidy, because the two conditions need not be entirely analogous. The discussion which follows will consider only cases of the latter type, to which the tomato triploid belongs.

Where the origin of triploids has been observed, they have either appeared suddenly as mutations, as one or a few plants in a diploid population, or they have been produced secondarily by crossing a diploid with a tetraploid which has so originated. The tomato triploid (LESLEY and MANN 1925) appeared as one unfruitful, somewhat gigantic plant in a plot of 90 normal ones. We do not yet know much about the means by which triploids originate, but they probably depend upon the origin either of tetraploid cells or of a tetraploid plant with subsequent fertilization of a tetraploid by a diploid germ cell. WINKLER (1916) reports the occurrence of a sterile tetraploid tomato following grafting of S. lycopersicum tissues on nightshade (S. nigrum), but no tetraploid plants have thus far been observed in Mr. LESLEY'S cultures. However, roots of diploid plants do sometimes contain large tetraploid portions (M. M. LESLEY 1925), and it would therefore not be impossible that occasional tetraploid gametes might be present. These plants have not vet produced tetraploid shoots nor have they been examined for occasional tetraploid germ cells, so that thus far the production of triploid plants is our only evidence that tetraploid germ cells occur. The sterility of WINKLER'S tetraploid plant is somewhat surprising since triploid plants are found to occur. Several tetraploid branches have been found on diploid Datura plants and these

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have produced tetraploid offspring (BLAKESLEE and BELLING 1924). L. V. MORGAN (1925) describes the occurrence of a tetraploid female Drosophila among the progeny of a triploid female. It was noted above that the chromosomes of the triploid tomato appear in diakinesis as twelve groups of three each, or as trisomes. This has also been found in the mosses and in Canna, Morus, Datura, Hyacinthus and Drosophila, but in the Oenothera triploids GEERTS (1911) found 7 pairs and 7 unpaired chromosomes, while GATES (1909) found no pairing or very loose pairing in the triploids, and a similar condition in the tetraploid (GATES 1911). METZ (1922) described parasynaptic union of the four homologous threads in Sarcophaga at diakinesis, but the figures given by WINKLER (1916) for the heterotypic metaphase of his tetraploid tomato show no tendency towards grouping into quadrivalents. The trisomes of Drosophila (METZ 1923) differ from those of Datura and tomato in that the former appear side by side in threes at the heterotypic metaphase, whereas the chromosomes of the trivalents of the two latter commonly remain attached by the tips only. The trisomes found in tomato are very similar to those described for Datura (BELLING and BLAKESLEE 1923). BLAKESLEE (in DAVENPORT 1924, p. 28) says,

"It has been shown that in haploid, diploid, triploid, and tetraploid Daturas, the chromsomes at the metaphase are joined in such a way that the homologous chromosomoes are in contact at the ends only. (This is not the case in some other plants such as Hyacinthus and Uvularia.) In the above mentioned Daturas the chromosomes are grouped in configurations which agree with the assumption that only corresponding or homologous ends come together".

The early prophase has not yet been described for the Datura triploids, but the similarity of the diakinesis stages in Datura and tomato indicate that it is possible that the earlier stages are also alike in these two Solanaceous plants. It appears, then, that all three chromosomes of a trisomic set probably pair side by side or parasynaptically in Drosophila, while in the tomato and possibly in Datura two of them unite parasynaptically, the third being attached either to the tip of one member of the pair or between their tips. The diploid tomato shows true parasynapsis, but in the triploid a parasynaptic union between all three chromosomes occurs rarely if ever.

The Diptera are outstanding in that even in somatic metaphase, homologues lie side by side. This association is even closer in the somatic prophase (METZ 1922). The somatic prophase of the tomato has not yet been carefully studied, but the homologous chromosomes seem, like those of most plants, to show no such "attraction" in the metaphase. Since synapsis is different in tomato and in Drosophila one would expect breeding results to differ in that crossing over between three sets of genes would be expected in Drosophila, but not in the triploid tomato. There is as yet no evidence on this point from the tomato, but crossing over between all the X chromosomes sometimes occurs in the triploid of Drosophila (MORGAN, STURTEVANT and BRIDGES 1924; L. V. MORGAN 1925).

The trisomes of Datura and tomato resemble the figures which show an X chromosome attached to an autosome (WILSON 1925, p. 776, figure 372). In diakinesis twelve trisomes are found in Datura and tomato, but at the metaphase each may show one to several univalents and a corresponding number of bivalents. The first division is far more regular in appearance than is usual in "triploid" species hybrids, in which, as for example in the 7×14 wheat species hybrid (KIHARA 1919), there are typically 7 pairs and 7 univalents at the first metaphase. The lagging of univalent chromosomes appears to be due to the fact that it takes longer for chromosomes to divide equationally than for them to assort. This difficulty is lessened for most of the chromosomes of the triploid by their trivalent condition. The lagging chromosomes of the first division are restricted to those few that fail to remain attached to their mates. The two chromosomes which have paired parasynaptically tend to remain together, the telosynaptically attached one tending to disjoin. This is suggestive of the observations on telosynapsis in Oenothera, in which pairing is sometimes so weak that it is difficult to distinguish chromosome pairs at the metaphase (DAVIS 1911). Observations on distribution of chromosomes in triploid pollen mother cells indicate that independent assortment of double or single chromosomes is the rule, at least in Datura, Hyacinthus and the tomato. The agreement of numbers in the different chromosome-number classes at the second metaphase, with the expectation as well as observations of lateral first anaphase, show that division of univalents is rare at the first division. As in Hyacinthus (BELLING 1924), the slightly greater number of counts in the lower number classes in the tomato is doubtless due to the greater ease in finding accurately countable figures with low than with high numbers. More laggards are to be seen in the second than in the first division in the tomato.

GATES (1925) states that triploids can perpetuate themselves only by apogamy or by becoming hexaploid. The triploid tomato is not apogamous, so that it can only be maintained by cuttings.

SUMMARY AND CONCLUSIONS

1. In the diploid tomato there is a true spireme and typical parasynapsis. GENETICS 11: My 1926 2. In the triploid the spireme consists, after synapsis, of a series of alternating paired and unpaired threads. The paired threads correspond to the parasynaptically paired strands of the diploid. No parasynaptic union between three threads was observed.

3. On the basis of the observations on synapsis in the triploid tomato one would not expect to find crossing over between all of these strands, although presumably it might occur between any two chromosomes.

4. At diakinesis there are usually twelve trisomes similar to those found in Datura, but univalent chromosomes are sometimes seen.

5. The chromosomes assort independently at the first meiotic division.

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