Microsporogenesis in Diploid and Triploid Types of Lilium tigrinum
with Special References to Abortions

By Clyde Chandler, W. M. Porterfield, and A. B. Stout

The several clones of Lilium tigrinum in general cultivation are known to have a somatic number of 36 chromosomes (Takenaka and Nagamatsu, 1930; Mather, 1935). The diploid number characteristic of other species of Lilium is 24 (See especially Strasburger, 1882; Guignard, 1884; Coulter, Chamberlain, and Schaffner, 1897; Allen, 1904; Satō, 1932; and Mather, 1935) and recently certain plants to be classed as Lilium tigrinum have come from Japan into culture in America which have a somatic number of 24 chromosomes (Stout, 1933). Satō (1932) mentions “wild” plants of L. tigrinum which produce seed but does not report that these or the seedlings grown are diploid. The several clones of Lilium tigrinum in culture which have a somatic number of 36 chromosomes are presumably auto-triploid and closely related in respect to origin.

Plants of the diploid type are smaller in stature than are the triploids; the flowers are smaller, their segments are narrower, and the spots on the petals and sepals are, as a rule, smaller. The pollen produced is highly viable and the various plants reared from seed are highly productive of seed when there is compatibility either in selfing or in cross-relations.

The triploid clones produce few pollen grains that are viable and in addition there is complete incompatibility for all intra-clonal and inter-clonal pollinations. There are then two distinct types of sterility in the members of the species Lilium tigrinum: (1) there are physiological incompatibilities in fertilization and (2) there are the abortions of spores which are frequent in the triploid clones.

The studies here reported are especially concerned with the various critical stages in the microsporogenesis of triploids with reference to the abortions which develop. Some comparisons are made with the various corresponding stages of microsporogenesis in diploid plants.

Material and Methods

Flower buds were collected from both the diploid and the triploid forms of L. tigrinum. Temporary aceto-carmine preparations were made from each bud. If the Pollen Mother Cells (P.M.C.) were at
the desired state of meiosis two of the remaining anthers were placed in some killing and fixing solution as Allen's modification of Bouin's, Flemming's medium, Carnoy's, or chrom-acetic. These were run through the paraffin method, embedded, sectioned on a microtome 24–30 μ thick, and stained with crystal violet, Heidenhain's haematoxylin, Flemming's triple, or Feulgen's. The other two anthers from the flower bud were placed in a solution of 100 c.c. glacial acetic +200 c.c. absolute alcohol for 24 hours and then transferred to 80% alcohol in which they were held until time for study was available. Belling's method for making aceto-carmine smears (Belling, 1926) proved most satisfactory for all stages except very early prophases and almost mature pollen grains. The earlier stages were more easily studied from the haematoxylin or the crystal violet preparations, while Ehrlich's haematoxylin stained the chromatin contents of microspores and microcytes most readily.

The Identity of the Individual Chromosomes

The two sets of 12 chromosomes each in the diploid *Lilium tigrinum* and the three sets of the triploid clones are remarkably alike in respect to the size and the shape of the individual chromosomes. For a triploid type Takenaka and Nagamatsu (1930) figured seven of the twelve chromosomes of a set and made the observation that one of the twelve has a median constriction and one a sub-median while the remainder have terminal or sub-terminal constrictions.

Satō (1932, p. 85) has studied the relative lengths and shapes of the chromosomes of various species of *Lilium* and he concludes as follows:

"The chromosome complement in each species of this genus is composed of elements which bear a striking resemblance in shape to each other, namely four long chromosomes which form two pairs of chromosomes with median and sub-median fiber-attachments, the second class being represented by three pairs of chromosomes with sub-median fiber-attachments, the third by rod-shape with sub-spherical head end, the fourth by the rod-shape with round end."

Satō's figures are of *Lilium tigrinum* clone Flore-pleno and were made from fixed material of somatic mitoses. Our studies were made from aceto-carmine stains of stages in meiosis for triploids which have the single flowers. We found that each of the 12 chromosomes of a set has an individuality in shape, form, and place of fiber attachment which is shown in the following text figure.

The set here shown is from the type clone of the triploid *Lilium tigrinum* which was first sent from China to the Royal Botanic Gardens at Kew, England, by William Kerr in 1804 and propagated since solely by asexual means for horticultural culture. The draw-
ings were made from unusually clear aceto-carmine preparations of the second anaphase, since the chromosomes at this stage are simple and easily studied. A summary of the characteristics of each chromosome is given in the following table:

Table 1. Tabulated description of the chromosomes of the type clone of the triploid *Lilium tigrinum*.

<table>
<thead>
<tr>
<th>Chromosome letter</th>
<th>Length in ocular units</th>
<th>Constriction point</th>
<th>Outstanding identifying marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11–7.5</td>
<td>Sub-median</td>
<td>Arm nearly as long as body.</td>
</tr>
<tr>
<td>B</td>
<td>9–6</td>
<td>Sub-median</td>
<td>Arm proportionately shorter; ends tend to curve inward; slight undulations.</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>Terminal</td>
<td>Long, slender, slight jog in the middle region; no knobs.</td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td>Terminal</td>
<td>Terminal knob; knob never bends over.</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>Sub-terminal</td>
<td>Slender, slightly undulate, both ends curved; small knob at polar end.</td>
</tr>
<tr>
<td>F</td>
<td>9.6</td>
<td>Sub-terminal</td>
<td>Terminal knob about 2 units in length. Always bent over.</td>
</tr>
<tr>
<td>G</td>
<td>9</td>
<td>Terminal</td>
<td>Undulate with definite median jog; no knob.</td>
</tr>
<tr>
<td>H</td>
<td>9</td>
<td>Terminal</td>
<td>Simple, straight with posterior end twisted obliquely to the side.</td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>Terminal</td>
<td>Posterior hook or right angle bend.</td>
</tr>
<tr>
<td>J</td>
<td>7</td>
<td>Sub-terminal</td>
<td>Small knob at polar end. Always bent over.</td>
</tr>
<tr>
<td>K</td>
<td>7</td>
<td>Sub-terminal</td>
<td>Large terminal knob bent over; body very undulate.</td>
</tr>
<tr>
<td>L</td>
<td>5</td>
<td>Sub-terminal</td>
<td>Short with straight body and terminal knob bent over.</td>
</tr>
</tbody>
</table>

Six of the twelve chromosomes, A, B, D, F, G, and K, are easy to recognize and the others are to be identified with a little more
study. In the triploid there is no apparent morphological difference between the homologues, but there are undoubted genetic differences and physiological differences as demonstrated by the irregular pairing of chromosomes and the appearance of univalents in the early stages of meiosis.

The chromosomes of the diploid as well as those of three clones of triploid *Lilium tigrinum*, i.e. splendens, Fortunei, and the intermediate type, were studied with respect to their individuality, but thus far no noteworthy differences between corresponding or homologous chromosomes of the different sets have been observed.

**Meiosis in the Diploid (2n=24) *Lilium tigrinum* Compared with That of the Triploid (3n=36) Clones**

The conspicuous cytological difference between the diploid tiger lily and the triploid type is the presence of the additional twelve chromosomes in the latter. As the stages in microsporogenesis are followed through in both types, it becomes at once apparent that the triploid P.M.C. display very definite irregularities in cytological behavior. Briefly they are as follows:

1. The presence of univalent, bivalent and trivalent spiremes in the prophase as well as the corresponding chromosome associations in the metaphase.
2. The distribution of more than 12 chromosomes to daughter nuclei at both I-A and II-A.
3. Lagging chromosomes at I-A and II-A.
4. Fragmentation of chromosomes and formation of microcysts at I-A and II-A.
5. The premature division of univalents at I-A.
6. The organization of micronuclei and their degeneration.
7. The formation of microcytes and the occurrence of polypory.
8. The abortion of a large percentage of the pollen grains and the ability of only a few grains to function.

In comparing the meiotic stages in diploid and triploid types of *L. tigrinum* we will trace these irregularities from their inception beginning with the post-synaptic phases.

**The Early Stages of Meiosis**

In the diploid type of *Lilium tigrinum* there is complete pairing of homologous chromosomes to the degree that no univalents were observed in these studies (plate 24 figs. 1 and 2). Immediately following synapsis there is a thickening and a shortening of threads and
it is soon evident that there are twelve paired threads of which the free ends are usually to be observed. The chromomeres are of various sizes and distribution along the spireme and they lie side by side in pairs giving a very complete and regular parasynapsis. From early pachyphase it is increasingly evident that a matrix is precipitating around the spireme threads forming a sheath. The sheath gradually acquires chromaticity and forms the bulk of each chromosome. In every nucleus there are one or two nucleoli which persist until late metaphase.

The chromonemata are first evident in the diplophase when they are observed to be twisted about each other. Each chromonema forms the axis of one chromatid (Taylor, 1931; Hsu-siang, 1932). Twisting of the chromatids initiates a coiling of the homologous chromosomes about each other which in this stage reaches its maximum (plate 24 fig. 3). A shortening of the chromatids, and consequently of the chromosomes, gradually takes precedence over coiling so that as diakinesis is approached actual untwisting begins to take place. While coiling is going on, however, there is a tendency for loops and loosely associated regions to appear between twists until shortening takes up the slack. Then the paired chromosomes resemble in appearance a segment of twisted rope until diakinesis brings another opening up of the chromatids. While the chromosomes shorten and commence to uncoil at the end of the diplophase, the chromonemata make no proportional changes. Increased coiling adjusts them to the decreasing length of the chromosome. In this respect the chromatids follow the behavior of the chromosomes rather than that of the chromonemata within. Sub-medial reversal of the direction of coiling of the chromonemata has been noted in both diploid and triploid P.M.C. (plate 24, fig. 3). This is common at the constriction point (plate 24, fig. 8) where the spindle-fiber attachment occurs (Iwata, 1935).

Sass (1934) describes the early prophases as being perfectly normal and implies that synapsis proceeds between pairs of homologous chromosomes. His figures, however, show irregularities in chromosome behavior which indicate that his material was not homogeneous, but was on the other hand derived from several different clones. It is possible that his material may have been collected from both diploid and triploid clones of *Lilium tigrinum* or that sufficient study had not been made of the early prophases to distinguish these irregularities.

*In the triploid*, irregularities are apparent in the late stages of synapsis in that there are univalent threads as well as bivalent and trivalent associations quite as noted by Horton (1936) in wheat.
The single or univalent spiremes (plate 24, fig. 5) may course independently through the nucleus without contact with other threads and will continue into the later stages as univalent chromosomes.

In the triploid, irregularities in the disposition and association of chromomeres on opposed homologous chromosomes were observed. Corresponding chromomeres on all three spiremes do not always occur and their sizes may be different as noted by Belling (1931). In shape the chromomere aggregates resulting from the conjugation of three homologous chromosomes are somewhat elongated and lobulated (plate 24, fig. 4) in contrast to the diploid in which the paired chromomeres are smaller and transversely lengthened (plate 24, fig. 2). As the prophase proceeds the chromomeres reduce in size and finally disappear, the smallest ones first.

The twisted condition of the three associated chromosomes in the diplophase produces bulky "rope" segments where all three are in conjunction, but the association in most instances and for considerable distances along these chromosomes is loose and irregular. Two spiremes are usually paired while the third may be in contact at one or two points only, and in some cases it may not touch at any point. It then constitutes an unpaired chromosome. Thus there are trivalent, bivalent, and univalent spiremes which originate at synapsis and carry on through diakinesis and metaphase (plate 24, figs. 4 and 5). Shortening of the elements and coiling of the chromonemata proceed normally (plate 24, figs. 3, 6, 8).

**Diakinesis**

In the diploid there are twelve pairs of chromosomes at diakinesis (plate 26, fig. 24). There are no unpaired elements. The chromosomes are shortened and the number of chiasmata in each pair is clearly evident. The individuality of the chromosomes can now be noted in respect to length, number of chiasmata, and the location of the constriction point. The components in each pair are opened out between the chiasmata and this forms characteristic nodes and internodes (plate 24, fig. 6). Each chromosome is composed of two chromatids which apparently contain one chromonema each. The contraction of the chromatids is responsible for progressive decrease in the length of the chromosome. With the decrease in length and the thickening of the chromatids the amount of twisting of the chromatids about each other decreases (Mather, 1935). While the shortening and straightening of the chromosomes is taking place, the chromonema in each chromatid increases its coiling since the chromonemata themselves do not shorten in length (Taylor 1931).

Mather (1935) noted in *Lilium* that chiasmata frequency is
proportional to chromosome length. We corroborate this conclusion from our observations of diakinetic chromosomes in the diploid *Lilium tigrinum*. The greatest number of chiasmata was observed in the pair of A chromosomes in which there were as many as four interstitial and two terminal. Two terminals were observed for E, F, J, and K; none were observed for the pairs of B, D, E, and H. One interstitial was the rule for G, H, I, J, and K, two for D, E, F, and L; three for C; and four for A and B.

![Diagrams showing chromosome associations](image)

**Text Fig. 2.** Semi-diagrammatic drawings (made to scale) showing configuration of the various chromosome associations at diakinesis in both the diploid and triploid *Lilium tigrinum*.

In the triploid *Lilium tigrinum*, the associations at diakinesis show three main types of irregularities: (1) lack of uniform association between the homologues, (2) chromosomes in different stages of shortening and straightening, and (3) the occurrence of unpaired chromosomes (plate 26, fig. 27). There are irregularities in the number of homologous chromosomes which synapse, and in respect to the reduction in the number of chiasmata and the straightening out of chromosomes the stage seems to be more advanced than in the diploid. Also the individual characteristics of the chromosomes are more evident; for example, the terminal ball on chromosome D is now clearly defined.

In respect to irregularity of association, two of the components are usually in closer contact with each other than with the third (plate 24, fig. 7). In text figure 2 the triploid associations for chromosomes A', B, C, E, G, I, and K illustrate this point. F, L, and J
show about an even degree of association between all three components. In the case of chromosomes A, D, and H the lack of association is evident from the fact that one component of each group frequently remains unpaired. In the unassociated chromosomes (univalents) the shortening and straightening apparently take place more rapidly than in those in which close contact is preserved (B, C, E and L). Definite association of more than three was not observed.

The condition shown for the D chromosome illustrates the behavior frequently seen for unpaired chromosomes. In this case all three of the D homologues are univalents and in each the two constituent chromatids have become not only evident but partially separated. The forces which operate to disjoin associated homologues seems to operate to develop and separate the chromatids of univalents.

**The Association of Chromosomes in the Metaphase of the First Division of Meiosis**

*For the diploid.* During the prophase and at the time of the equatorial plate there are 12 pairs of homologous chromosomes. No unpaired chromosomes were observed. Two closely coiled chromonemata may be observed in each chromosome. Twisting of the members of a pair may be seen especially for the pair of the largest chromosomes (A and B) in which case there are points of contact some of which may be true chiasmata (plate 24, fig. 6), or cases of false interlocking. The associations observed at metaphase originate during synapsis. Several cases of interlocking have been noted in this connection (text fig. 2, triploid K) but the twists and coils have undergone reduction until at the time of maximum contraction no more than two twists or one complete coil remain in the longest chromosomes, while the smaller chromosomes have fully straightened out. But at this stage coiling of the chromonemata reaches its maximum in number and compactness. Premature disjunction of chromosomes or the premature separation of chromatids has not been observed for the diploid.

*For the triploid.* Univalents, bivalents, and trivalents are in evidence during the late stages of prophase and in the equatorial plate of the I-M, but it is seldom that all the chromosomes of a triploid P.M.C. are associated in 12 groups of three each. The associations determined for 98 P.M.C. from one anther were as follows:

<table>
<thead>
<tr>
<th>Number of trivalents</th>
<th>12</th>
<th>11</th>
<th>10</th>
<th>9</th>
<th>8</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>2</td>
<td>29</td>
<td>27</td>
<td>25</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
Takenaka and Nagamatsu (1930) reported that in the triploid *L. tigrinum* which they studied the number of trivalents ranged from 6 to 12 with the greatest frequency for 8. Our data indicates that the greatest number of trivalents is much higher than 8.

The associations of the long chromosomes (as A, B, and C) in trivalents present various configurations (text fig. 2, triploid A', A) which are no doubt the effect of differences observed earlier in the synopsis of parts of the threads or the results of chiasmata. The irregularities in the triploid at this stage involve numerical differences in the number of chromosomes which synapse and the configuration of those which are trivalent. In the trivalents there is contact at certain points between any one and the other two, but it is often the case that two are rather closely associated while the third is only partially bound to the others (plate 24, fig. 9). Complete terminalization of chiasmata proceeds up to the beginning of the anaphase but the high viscosity and cohesive qualities of the sheath preserve contact associations beyond that point.

The univalents, bivalents and trivalents are all brought into the level and plane of the equatorial zone (plate 26, fig. 27) and in this particular there are no lagging elements at this stage. The nuclear membrane soon disappears while at the same time the sheath of the chromosome increases in volume and its outer surface becomes more dense. The nucleolus loses its chromaticity at this point and its outline becomes irregular as though parts of it had already undergone partial dissolution. Owing to extreme contraction the individuality of the chromosomes is somewhat obscured.

![Text Fig. 3. Semi-diagrammatic drawing made of a diploid P.M.C. of *Lilium tigrinum* at I-A showing the entire set of 24 chromosomes with partially separated chromatids evenly distributed between the two poles. (From an aceto-carmine smear preparation).](image1)

![Text Fig. 4. Semi-diagrammatic drawing made of a diploid daughter cell of *Lilium tigrinum* at II-A showing the entire set of 24 daughter chromosomes (former chromatids) evenly divided between the two poles. (From an aceto-carmine smear preparation).](image2)
The Anaphases of the First Division

In the diploid the pairs of homologous chromosomes disjoin with regularity and one set of twelve moves to each pole (plate 26, fig. 25). As the chromosomes proceed, partial separation of chromatids simultaneously takes place so that each of the polar groups is composed of twelve pairs of chromatids. There is no precocious complete separation of chromatids. The chromosomes with terminal and subterminal spindle attachments appear as V-shaped structures (text fig. 3), as shown by Farmer (1895) and later by Allen (1904), with the apex pointed toward a common center, the two chromatids being in contact and bound together only at the point of fiber attachment. The long chromosomes with sub-median attachments approximate double V's with the four ends divergent and the angles coinciding at the constriction region in a bond between each two chromatids. Each chromatid definitely contains now two coiled chromonemata as duplication has taken place. For the entire cell at this stage there are twenty-four pairs of chromatids. There are no lagging chromosomes; and the spindle is bipolar without exception.

In the triploid clones of Lilium tigrinum there is in the first division (1) complete disjunction for nearly all members of bivalent and trivalent associations with only an occasional non-disjunction of an homologous pair of chromosomes, (2) partial separation of certain sister chromatids from each other, (3) complete separation of certain of the sister chromatids especially those of the univalents, (4) the lagging of certain chromatin units, and (5) an unequal numerical distribution of chromatin units to the two poles. The rule is that in the anaphases of the triploid types the homologous chromosomes disjoin and also that the two chromatids of each of these chromosomes partially separate, while the chromatids of univalents entirely separate to form the sister chromosomes which would otherwise normally appear in the second division (plate 1, fig. 10).

When they are disjoining, the chromosomes C, D, E, F, G, H, I, J, K, and L pass to the poles as open V's while chromosomes A and B appear as double V's with coinciding vertices and the appearance of these is the same as in the diploid (plate 3, fig. 34). As a rule the unpaired chromosomes (the univalents) fail to leave the equatorial plate and their chromatids completely separate in the equatorial region (plate 3, figs. 28, 29, 30, and 31).

Complete counts were made in various cells with the definite identification of the pairs of attached sister chromatids (diads) and of the separated chromatids (monads) with respect to the distribution in the anaphase of the first division of meiosis. The different distributions observed are given in the following tabulation:
Table 2. Summary of chromosome distribution at I-A in P.M.C. of triploid *Lilium tigrinum*.

<table>
<thead>
<tr>
<th>At one pole</th>
<th>Lagging</th>
<th>At second pole</th>
<th>Total equal to</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 diads</td>
<td>10 monads</td>
<td>15 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>17 diads</td>
<td>8 monads</td>
<td>16 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>17 diads</td>
<td>6 monads</td>
<td>15 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>19 diads</td>
<td>4 monads</td>
<td>17 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>17 diads</td>
<td>4 monads</td>
<td>16 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>19 diads</td>
<td>2 monads</td>
<td>17 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>18 diads</td>
<td>2 monads</td>
<td>18 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>18 diads</td>
<td>0 monads</td>
<td>14 diads</td>
<td>36 diads</td>
</tr>
<tr>
<td>20 diads</td>
<td>1 diad and</td>
<td>14 diads</td>
<td>36 diads</td>
</tr>
</tbody>
</table>

In one of the mother cells listed above one nucleus had 14 chromatin units, the other had 20, and there were 3 lagging, making a total of 37. All the units in the two polar groups were pairs of chromatids (diads). One lagging unit was a V-shaped diad of the chromosome K, but the other two were monads (separated chromatids). Hence the total of 37 is in reality the equivalent of 36 diads. The presence of monads or separated chromatids to the number of 10, as indicated in one case above, gives a total of 41 chromatin units which, however, are equal to the 36 chromosomes of the triploid assembly.

In the case of the distribution of 20 and 14 diad chromosomes noted above, it was determined that at one pole of the 20 there were five pairs of duplicates and of the 14 there were three pairs of duplicates, and in each of these, two homologous chromosomes had passed to the same pole but the pairs were not attached to each other.

The data indicate that the mechanism of distribution operates most effectively for the disjoining of chromosomes and that it tends to separate these into two groups of nearly equal number. Since the number at each pole is nearly always more than 12 it is evident that there is the inclusion of two or more chromosomes of the same type (homologues) within the same polar group. It may, perhaps, be assumed that two of a trivalent group tend to pass to opposite poles and that the third member may go to either. In the counts which were made no case was found in which only 12 chromosomes were found at one pole of the anaphase of the first division, and in no case was 24 found.

The lagging chromatin elements in the early anaphase stages of the first meiotic division in triploids are either (1) pairs of homologous chromosomes, (2) unpaired chromosomes, or (3) single and separated chromatids. The last named are most frequent and numerous. Failure of disjunction sometimes occurs. In this case the pair of homologues may be stretched to form a chromatin band.
which extends for a considerable distance toward the two poles. If there is a persistent attachment in the distal portion of the two, an "anaphase bridge" (Jensen, 1936) is formed which is later bisected by the formation of a cell plate.

Unpaired chromosomes which lag rarely persist in the equatorial areas as such; that is, with the two chromatids intact in one unit or in partial attachment. It is the rule that the lagging univalents undergo premature separation of chromatids which is the final stage of duplications normally seen in somatic divisions and in the homeotypic divisions of meiosis.

The position and condition of the separated chromatids in the early anaphases indicate certain aspects of the processes which operate. The two sister chromatids separate promptly and completely during the early anaphase and for a time they lie near each other in a somewhat parallel position. They seem to float apart by a repulsion which is operating uniformly at all points including the point of spindle attachment. The separation appears to be largely independent of spindle fiber activity and the operation of such fibers seems to be largely lacking on the separated chromatids in later stages.

Chromosomes A, B, D, E, and K were all identified among the lags but it can not definitely be stated that certain chromosomes of the twelve do not lag. It is, however, certain that the chromosomes which most commonly lag are those for which associations are loose or lacking (as illustrated by chromosome D).

The spindle figures of the first division are bipolar and there is no evidence of the suppression or collapse of the general spindle apparatus which might result in restitution nuclei. All chromatin elements and associations seen in diakinesis are brought into the metaphase plate. From this point on the distribution operates to distribute into two nearly equal groups the chromosomes which have been associated and which have disjoined, but fails thus to operate on
those univalents whose chromatids separate prematurely to form sister chromosomes. The only noticeable irregularity in spindle formation is the appearance of the secondary or accessory spindles which form cell-plates to cut off microcytes (plate 25, figs. 21, 22, and 23).

The Telophase and the Interphase following First Division

The points of special interest in these stages are the organization of the main nucleus in each of the two cells and the behavior and fate of the lagging chromosomes and the fragments of chromatin.

The group of V-shaped pairs of chromatids at each of the two poles (plate 26, fig. 34) is oriented in a manner which suggests the so-called maturation repulsion forces postulated by Takenaka (1933). The individual pairs of chromatids in both diploid and triploid *L. tigrinum* are somewhat elongated during traction toward the pole indicating tension, but as the polar position is reached the shape becomes more undulate. The group of chromosomes, as a rule, is at first star-shaped and somewhat flattened in one plane. But soon the nuclear membrane appears and the plate of chromosomes becomes bent in conformity to its curved surface. No cytoplasm appears to be included within the nuclear space which becomes spherical in shape. Concurrent with the organization of the nuclear membrane the sheath of the chromatids loses volume and practically disappears disclosing thereby the spiral chromonemata. Each chromonema has duplicated and in most of the chromatids the chromonemata have actually separated giving the characteristic criss-cross of the double spiral. In some regions among the chromosomes the chromonemata may still be seen unseparated (text fig. 5). The chromosomes retain their V-organization the ends of the chromatids remaining divergent. They elongate and become tortuous but there is no tendency evident of the free end of one "V" fusing with that of another as suggested by Allen (1904). Lengthening of the chromosomes is evident with the spinning out of the coils (plate 25, fig. 23). No nucleolus is organized and no anastomoses between chromatids are seen.

While the interkinetic nucleus is being organized, the phragmoplast is expanding toward the periphery and any fragments and lagging elements not included with the polar groups at the time the nuclear membrane appears are swept along to the periphery and remain there either to undergo degeneration or else, if in sufficient quantity, to organize as a micronucleus. A small and secondary spindle may arise in connection with the formation of the dividing wall of a microcyte (plate 25, fig. 23). In the diploid no such extruded elements are present. Following the contractions of the next pro-
phase, the chromonemata coil anew and accumulate matrix thus bringing into existence the new chromatids. No chromomeres have been observed at this stage.

The lagging chromosomes and the fragments of segmented chromosomes which are a conspicuous feature in the triploids (plate 25, figs. 19 and 20) are, as a rule, left outside of the newly organized nucleus. The fragments, and even entire chromosomes (plate 25, figs. 12–17) soon become rolled up into compact balls and there is increasing opacity, accompanied by the development of one or two lymph vacuoles which are first in contact with the fragment (plate 25, fig. 11a, b, c, d, e, f, g, h, i). We propose to call the rolled-up chromatin masses which do not become organized as a nucleus or which later appear from disintegrating micronuclei, microcysts (plate 26, figs. 32 and 33). Takenaka (1933) calls such bodies “chromatin granules” and Täckholm (1923) calls them “nucleoli” and illustrates them in his figure 33. It appears that the vacuoles separate from the microcysts (plate 25, figs. 11f, g, h; 18 and 21) and migrate during the interphase toward the nucleus, for cases were observed in which several of them were close to the periphery of the nucleus. The mode of formation, the appearance, and the behavior of these vacuoles suggest that they are formed by the complete dehydration of the chromatin, and that they contain true karyolymph.

Certain chromatids and as many as three chromatids which lag are organized into micronuclei which lie free in the cytoplasm. These may persist but they do not undergo a second division.

Secondary spindles and microcytes. At the end of the first meiotic division of the triploid P.M.C. secondary spindles may develop either when cell-plate formation is still in progress or soon thereafter. One pole of such a spindle centers about the lagging chromatin which has become localized in the periphery of the daughter cell following cell-plate formation. The other ends indefinitely in the cytoplasm in the general direction of the nucleus, the fibers converging and disappearing (plate 25, figs. 21, and 22). The cell-plate formed cuts off a dwarf cell termed a microcyte (Hollingshead, 1930) (text figs. 7b and c, 8; plate 26, figs. 35, 36, 37). These are formed by secondary spindles and they originate after the daughter cells have separated. Accessory spindles, on the other hand, occur as an extension of or in conjunction with the phragmoplast during the process of cytokinesis (plate 25, fig. 23). One pole centers about the localized chromatin which may or may not organize a micronucleus, while the other pole is diffuse, some of the spindle fibers proceeding toward one of the nuclei, the others toward the second nucleus. The cell-plate which results appears to be bifurcated.
or branched, and the cytoplasm of the microcyte formed comes from
the original cell rather than from either of the daughter cells. The
chromatin undergoes no movement with either type of spindle, and
no evidence of traction or attempted distribution of chromosomes has
ever been observed. The sole function of such spindles is cell-plate
formation and they may occur in either the first or second divisions
of meiosis.

The Second Meiotic Prophase and Metaphase

Following the brief interphase in which the chromonematal coils
spin out to their limit, a second contraction occurs and the chromo-
somes of both the diploid and the triploid come out of the inter-
phase exactly as they entered it, that is, as diads. At this stage no
chromomereres or anastomoses are in evidence.

In the diploid there is a second division in each of the two
daughter cells and these are simultaneous but independent (plate 26,
fig. 26). Four spores of nearly equal size are formed and the primary
nucleus of each receives, as a rule, 12 chromosomes. No extruded
or lagging chromatin elements were observed in the various seedlings
which are to be classed as normal diploids. The chromosomes
assembled at each pole are the original chromatids and each one is
simple: that is, the limbs of the V's have separated (text fig. 4).

In the triploid there is also very uniformly a division for each
daughter nucleus and its cell (plate 26, fig. 33). But these nuclei
almost always contain more than 12 chromosomes some of which are
homologues and also there may be present in the nucleus certain
single chromatids. The developments in each of the two main cells
involve the separation of the chromatids of those chromosomes which
have remained as diads, the distribution of all the chromosomes
originating from single chromatids, the organization of these into
nuclei or as lagging elements. In addition there are the changes to
consider which take place in the micronuclei and microcysts which
are included in the two daughter cells from the first division of
meiosis.

At the first telophase it is evident from counts that some of the
chromatids which constitute the components of equationally divided
univalents are included in daughter nuclei. These are to be recognized
as monads instead of diads. As the reduction of the matrix proceeds
and the extending of the spiral chromonemata continues at inter-
phase the connection between the divergent chromatids can readily
be traced. This attachment point can be identified in the diads but
in the monads it is absent or less readily observed.

The second division gives, as a rule, from each pollen mother cell
four cells of nearly equal size, and in addition there may be formed
at this time smaller cells (microcytes) which may contain lagging chromosomes. These become organized as micronuclei or as microcysts, or they may contain only cytoplasm (plate 26, figs. 35, 36, and 37). The latter are in addition to any microcytes that may form during the first division.

The distribution of chromosomes during the second division was fully determined in 76 cases for each of the daughter nuclei of the four large cells (future pollen grains) and for the lagging elements, and in various cases the identity of certain chromosomes was established. The various distributions observed are given in the following tabulation in which the numbers given refer to chromosomes formed from single chromatids:

**Table 3. Distribution of chromosomes in the second or homeotypic division of *Lilium tigrinum*.**

<table>
<thead>
<tr>
<th>Total number</th>
<th>In the sister spindle</th>
<th>In one spindle</th>
<th>One nucleus</th>
<th>Lags</th>
<th>One nucleus</th>
<th>Lags</th>
<th>One nucleus</th>
<th>Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>1</td>
<td>18</td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>2</td>
<td>17</td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>17</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>2</td>
<td>18</td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>19</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>19</td>
<td>19</td>
<td>15</td>
<td>15</td>
<td>1</td>
<td>14</td>
<td></td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>16</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td></td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>15</td>
<td>15</td>
<td>2</td>
<td>14</td>
<td></td>
<td></td>
<td>67</td>
</tr>
</tbody>
</table>

The lagging chromosomes or fragments from the first division are now either rounded up as microcysts, tightly knotted, in advanced stages of disorganization, or disintegrated entirely, or they may be organized in micronuclei. They may be present in these conditions within the larger cells or in the microcytes. In table 3 the number of chromosomes in any one complement of the four large daughter nuclei ranged from 14 to 19. In several cases 72 chromosomes are counted which indicates that each chromosome of each of the three sets had undergone but one equational division even though this occurred in the first division. When the number is less than 72 either some chromosomes did not thus divide or else certain of them were lost through lagging and ultimate disintegration.

The identification of all chromosomes in a late anaphase of the second division was made with respect to the distribution of chromosomes to the four nuclei in a certain P.M.C. of the triploid *L. tigrinum*. In this case there were but three lags evident and these were from the last division. Text figure 6 presents a drawing which shows all the chromosomes and table 4 summarizes their distribution to the two pairs of sister nuclei.
It is evident that in this case all chromosomes divided once only; that more than a complete set have passed to each of the four nuclei; and that two of the three homologous chromosomes enter either of the two nuclei of a pair by chance distribution to increase the number to more than 12.

A special study was made of the number of lagging and extended chromatin elements in a large number of cases in which the number of chromosomes in the main nuclei could not be determined. The following table presents the various kinds of distribution of lagging elements observed in each of the two pairs of sister spores and in the additional microcytes. The lagging elements of the first division are given in italics, those of the second division are given in the ordinary type. In certain cases the identity of a chromosome was established as indicated in parentheses.
Table 5. Data on lagging chromatin units at the anaphases and telophases of the second meiotic division of the triploid *Lilium tigrinum*.

<table>
<thead>
<tr>
<th>Lagging in the two pairs of main cells</th>
<th>Data for microcells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A A'</td>
<td>B B'</td>
<td></td>
</tr>
<tr>
<td>2+1</td>
<td>1+1</td>
<td>1+2</td>
</tr>
<tr>
<td>2+2</td>
<td>2+2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3+1</td>
</tr>
<tr>
<td>3</td>
<td>1+1</td>
<td>2</td>
</tr>
<tr>
<td>1+1</td>
<td>1+1</td>
<td>1+2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>I (D)</td>
<td>I (B)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2+2</td>
<td>2+2</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1 (A)</td>
<td>1+1 (D)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>I (D)</td>
<td>I (F)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2+2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* f signifies fragment.

In the groups of cells reported above there was only one tetrad with no lagging chromatin units. The greatest number of such units was 18 in which 7 microcytes were present comprising 9 chromatin elements. The largest number of lagging chromatin elements in any one cell was 4. At this time the lagging units consisted of (1) entire chromosomes of which A, B, C, D, E, F, and H were identified as indicated in the table, and it will be noted these were all lagging from the second division, (2) chromosomes which were rolling up and forming irregular knotted masses, (3) fragments some of which had degenerated into rounded and apparently solid masses, and (4)
chromosomes in micronuclei. Some of each of the last three classes as mentioned were found in microcytes.

**Late Stages of Microsporogenesis**

The conditions which exist in the triploid *Lilium tigrinum* at the time of the late stages of microsporogenesis after meiosis is completed and shortly before anthers dehisce may be listed and briefly summarized as follows:

1. More than four main spores are frequently present, but never less than this number (plate 26, fig. 36). The four sister cells (the potential pollen grains) are rather uniform in size and shape and of larger size than the accessory microcytes (plate 26, fig. 35). There are some differences in size but all contain cytoplasm and one large nucleus, and there is usually present one or more lagging chromatin elements.

2. As a rule the number of chromosomes in the nucleus of each of the four larger cells derived from a single pollen mother cell is more than 12. The numbers counted at this stage ranges from 14 to 19, but the number of 12 has been found in the equatorial plate of the division of the vegetative nucleus of a microspore (text fig. 9). The organization of nuclei with more than 12 chromosomes seems to be the usual procedure.

3. In the cytoplasm of the large-sized or primary cells there are (a) degenerating chromosomes or fragments in the form here described as microcysts (text figs. 7 and 8, Mst.), (b) lymph vacuoles which arise in the formation of microcysts (text fig. 7, L.V.), and (c) micronuclei (text figs. 8 and 10, Mn.); and some of each of these may arise in the first division. At least 75% of the mature pollen grains contain one or more microcysts, the largest number observed in any one cell being eight. The degeneration of the lagging or extruded elements of chromatin proceeds both when they lie free in the cytoplasm and when a nuclear membrane is formed.

4. The micronuclei contain few chromosomes or chromatin fragments. Those which lie in the cytoplasm of a primary cell are reduced to microcysts (plate 26, fig. 38) and hence very few ripe microspores contain micronuclei.

5. Accessory cells or microcytes are present and conspicuous at this time (text fig. 7, b and c; 8, Mct.). They are much smaller than the primary cells; some of them are formed during the first division, but most of them are formed during the second division. For the most part they are formed after the main cell plates are fully developed. They may contain micronuclei, or microcysts, or both; or there may be no chromatin in evidence.
At the time when anthers of the triploid *Lilium tigrinum* are about to dehisce and discharge pollen there are two rather distinct classes of spores, (a) the large-sized pollen grains and (b) the small-sized microcytes and these exist in a ratio of about 9 to 1 (plate 26, fig. 39). All these spores contain at least some cytoplasm and have well formed walls. None have collapsed and none appear to be dead.

**The Mature Pollen**

During the last few hours before the anthers dehisce and during the early stages of dehiscence while pollen is drying out noteworthy changes occur and obvious degeneration followed by abortion of entire cells appears for the first time. Certain of the large-sized pollen grains do not develop beyond the one-celled stage and these, it is believed, not only do not germinate but abort. It is to be noted
that about 10% of the large-sized pollen becomes empty during the stages of dehiscence (plate 26, fig. 42).

For a considerable number of the large-sized grains the division of the primary cell occurs, but various irregularities in the internal development of pollen grains have been found.

1. The division of the primary cell may result in two cells of nearly equal size (See text fig. 11). This condition was shown by Chamberlain (1897; his figures 19 and 20). The two cells may contain no microcysts or micronuclei and appear as living and quite normal vegetative cells within a common microspore wall.

2. There may be present in the cytoplasm of the pollen grain microcysts and accessory micronuclei in various conditions and most of these arise during meiosis as already described. But in some cases there may be amitosis of the primary nucleus quite as shown by Chamberlain (his figures 13 and 14) which results in small nuclei similar in appearance to the micronuclei formed about lagging chromatin.

3. The generative cell may remain of small size and stay outside of the vegetative cell. It may become separated from the vegetative cell by a wall and may become more or less flattened and aborted (plate 26, fig. 41). This condition was shown by Chamberlain (1897) but the generative cell was interpreted to be a prothalial cell and a micronucleus within the primary cell was interpreted as the generative cell. The structures considered as centrosomes by Chamberlain are, we believe, microcysts in the late stages of degeneration.

4. A large number of the microcysts shrivel into irregular shapes and their contents collapse and disintegrate. Also in the drying of pollen, many of the larger grains become shrivelled to various sizes. Thus the dry pollen, either as such or after it has been placed on sugar-agar media, presents considerable gradation in size, degree of irregularity in shape, and density of contents even for those cells which previously appear to be rather uniform.

The irregularities found in pollen grains may be listed as follows:

1. One vegetative nucleus + one microcyte.
2. "   "   " + two micronuclei.
3. "   "   " + four microcysts.
4. "   "   " + two microcysts + a generative cell.
5. Two " nuclei.
6. "   "   " + one generative nucleus.
7. Three " "
8. Two " cells in one grain which may or may not be of equal size (text fig. 11).
The Viability of Pollen

It has been reported (Stout, 1933) that a germination of about 9% was obtained for pollen of Lilium tigrinum clone Fortunei which is triploid and that pollen of certain plants of this species which are evidently diploid gave 90% germination. Further tests for germination have given results fully in agreement with this earlier report.1)

Pollen of diploid in tests for artificial germination on agar (1%)-sugar (5, 10, and 15%) media gave germination for as many as 90% of all grains and only about 1% of all grains was empty and shrivelled. Many of the tubes reached a length of 2000 μ and some were 3000 μ in length (plate 26, fig. 43).

Pollen of the triploid clone Fortunei was rather fully tested and studied in artificial germination. Microcytes constituted about 10% of all cells to be included as spores and these were empty or nearly empty and gave no germinations (plate 26, fig. 40). There were also mostly empty and shrivelled cells to the number of about 10% which are larger than microcytes and which are certainly tetrad cells whose cytoplasmic contents have died and have largely disintegrated. Careful counts indicated that a germination of nearly 8% of all grains was obtained and the longest tubes observed were 2300 μ in length. About 70% of all grains did not germinate but did possess cytoplasmic contents either alive or dead which stained conspicuously with aceto-carmine. Tests with aceto-iodine showed the presence of starch. In nearly all cases a vegetative nucleus was observed and in many cases a generative cell was present either inside the vegetative cell or without.

Branscheidt (1930) reports that pollen of Lilium tigrinum which does not germinate in sugar culture may be stimulated to a germination of 61% by mixing the pollen with pollen of Helianthus and to a germination of 80% by the addition of diastase. The method of culture with diastase used by Branscheidt was employed and also various modifications of it were tested extensively at The New York Botanical Garden for pollen of several types of triploid Lilium tigrinum. The increased germination reported by Branscheidt was in no case obtained. Branscheidt does not state what type of Lilium tigrinum was studied by him. In the triploid pollen which we have studied at least 20% (including the microcytes) was empty and unable to germinate under any conditions. A considerable portion of the grains which did not germinate had cytoplasmic contents, but in many of these, various abnormalities were observed which together

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1) The earlier germination tests were made by Miss Olga Schweitzer and those of later date were made by Mr. Victor Shortino, in the Laboratories of The New York Botanical Garden.
appeared to restrict germination. At any rate in all of our many tests the germination of pollen of triploids has been low (about 8 or 9%) on the same sugar-agar media which give a germination for 90% of the pollen of diploid plants.

Considerable study has been made to determine the internal conditions which may exist in pollen which germinates. Such grains are of the larger size; they possess a thickened wall; their cytoplasm obviously remains alive; there is present a vegetative cell and a generative cell which is within the cytoplasm of the vegetative cell. In certain of the pollen tubes some microcytes and even micronuclei have been observed. The number of chromosomes in the nuclei of germinating pollen can rarely be determined by direct counts and our data on this point are meagre. In one case 12 chromosomes (see text figure 9) were definitely counted during the division of the primary cell, although it cannot be said that the 12 comprised one of each type. It may however, be assumed that microspores whose vegetative and generative nuclei contain a single but complete complement of 12 chromosomes are most likely to function in germination.

Summary and Conclusion

It is evident that the abortions of the microspores in the triploid clones of *Lilium tigrinum* arise in connection with the presence of an extra set of chromosomes. But the three sets function normally in the somatic tissues of the triploid plants in respect to cell divisions, organizations of nuclei and cells, growth, and differentiation; and they have so functioned in countless numbers of the vegetatively propagated plants of the triploid type over a period of at least 130 years. At the same time in the more intricate processes of meiosis, abnormalities develop and conditions arise which do not occur in the diploid. In the triploid these lead to abortion of pollen grains and the inability of many apparently living grains to germinate.

The abnormalities which can be observed during microsporogenesis appear (1) in various stages of the association or conjugation of the three homologous chromosomes, (2) in the premature separation of the sister chromatids in unpaired chromosomes, (3) in the distribution of chromosomes, (4) in the organization of cells which constitute the complement of spores and (5) in the internal development which produces mature and normal pollen grains.

The association of three spiremes is attempted and in large degree effected for various trivalents in every spore mother cell of the triploid type of *Lilium tigrinum*. Evidently the three homologues for each type of chromosome are able to associate in some degree, but partial associations are frequent and also entire single threads
appear in synapsis, which continue thereafter as univalents. The greater number of chromosomes enter into association as trivalents or bivalents and there is no evidence of larger groups which suggest secondary associations. This with the data that the characteristic chromosome number for species of *Lilium* is $2n = 24$ and the fact that no $4n$ species is known seems to indicate clearly that the triploid clones of *L. tigrinum* are autotriploids. The failure of complete association of all spiremes in sets of three is a feature of irregularity which contributes to certain later abnormalities.

The mechanism for the distribution of chromosomes both in the first and in the second division of meiosis is definitely bipolar. Its operation in the first division is to separate the chromosome associations, whether bivalent or trivalent, and to assemble them into two groups of nearly equal number. The only indication of a third group is seen in the lagging chromosomes which first lie in the bipolar spindle: they are left out of the main nuclei and are scattered; and there is no mechanism which can assemble them into a third group or that can operate earlier to place each of the various trivalent groups into three different nuclei. There are, however, accessory spindles which develop feebly as somewhat unipolar figures with one pole centering about lagging chromatin which happens to lie on or near the margin of the plate that is forming. Such spindles operate to produce only microcytes. There is no attempt to assemble or move the several chromatin elements to or from the pole where the nucleus may or may not be organized.

It is believed that the repulsion of the members of bivalent and trivalent associations in the triploid *Lilium tigrinum* is very complete and regular although nondisjunction does sometimes occur. Two homologous chromosomes are frequently included in the same nucleus although they have disjoined. The lagging chromosomes of the first division are chiefly univalents and these undergo an equational division which is premature.

The organization of two daughter cells in the first division and of two pairs of sister cells in the second division proceeds with a remarkable degree of precision and each of these contains a fully organized nucleus which nearly always has more than 12 chromosomes. In addition there may be microcysts and micronuclei in the cytoplasm. The organization of microcytes in both the first and the second divisions occurs as a result of lagging chromatin.

The irregularities in the distribution of chromosomes result in (1) lagging elements of chromatin, (2) the distribution of more than 12 chromosomes to the main nucleus of nearly all large-sized pollen grains, which necessarily involves the presence of one and
often two homologues of certain chromosomes or of two sister chromatids.

The lagging chromosomes contribute (1) to the incomplete distribution of chromatin between the daughter nuclei in both the first and the second divisions, (2) to the premature separation of univalents, (3) to the formation of microcysts and micronuclei many of which disintegrate in the cytoplasm, and (4) to the formation of microcytes.

The abnormal developments which may actually be observed within the pollen cells involve the following:—

1. The primary nucleus may fail to divide to form a generative cell or several micronuclei may arise from it by amitosis.

2. The division of the primary cell may result in two cells which may or may not be nearly equal in size (see text figure 11), neither of which can be considered as a generative cell.

3. The generative cell may be produced by a normal mitosis and the typical unequal division of cytoplasm but it may fail to enter the cytoplasm of the vegetative cell, in which case it often shows some disintegration.

4. The generative cell may enter the vegetative cell but fail to divide, or its nuclear material may form into more than two nuclei.

5. The lagging chromatin elements in the cytoplasm undergo noticeable and rapid disintegration. Microcysts form directly or by degeneration of micronuclei and later they disappear.

The conditions which are to be recognized as directly contributing to the death and abortion of the various classes of pollen grains as well as to their inability to function are as follows:—

1. A decrease in the total chromatin content which gives less than a normal haploid set of 12. This is especially seen in the micronuclei of microcytes in none of which there are more than two or three chromosomes and usually less. The contents of such cells disintegrate quickly during anthesis and no such cells germinate.

2. An increase of chromosomes in nuclei of large grains above the normal haploid number of 12. The evidence is that an even 12 chromosomes are seldom obtained.

3. Certain of the microspore cells abort and their contents die during anthesis. In these it is certain that one or more of the conditions noted above (1, 2, 3, 4 and 5) may exist and contribute to the abortions. It is observed that in the late stages in the abortion of these cells disintegration of the chromatin content of the nucleus may be observed.

4. Many microspores in which one or more of the final stages of internal development does not occur (1, 2, 3, 4 and 5 above)
appear to be alive although they may not germinate. The cytoplasm and the nucleus of the primary vegetative cell appears to be quite normal and intact.

The abortions of pollen may be attributed to conditions within the nuclei, to conditions in the cytoplasm, or to the combined action of both. Within the nuclei of the microspores there are as a rule more than one set of chromosomes, or at least more than 12, but as far as our observations go it is doubtful if there are ever as many as 24 and we find no evidence that restitution nuclei may form. The chromosome complement may, hence, be unbalanced in number, in genic complement, and in physiological processes. In the cytoplasm the obvious source of possible disturbance is the disintegration of chromatin material. This begins to occur in the telophase of the first division and progresses in degree and extent with the addition of more lags in the second divisions through the last stages of sporogenesis and even into pollen tubes of germinating spores. These disintegrations may, perhaps, be assumed to be toxic or to exert other unfavorable influence on the cytoplasm, on the chromatin within nuclei, or on both, which inhibits proper development of pollen.

Thus in the triploid *Lilium tigrinum* the presence of a third set of chromosomes in a meiotic mechanism which operates to produce two groups of nearly equal number gives irregularities in the mechanical redistribution of these three sets which seldom realize the normal haploid sets. These bring about definite disturbances in the organization of cells and nuclei and in the development of spores. The final abortions and death, as well as certain failures in germination, are to be regarded as internal for each cell or microspore. The organization of the nuclei in respect to kind and number of chromosomes and the disintegration of chromatin in the cytoplasm are to be regarded as the chief active influences.

The abortions of spores in autotriploids, as in *Lilium tigrinum*, in various other unbalanced polyploids, and in many F1 hybrids constitute a rather definite type of sterility which is distinct from similar abortion in unisexualism, and especially in diploids where definite pollen lethals of individual genetic value may be determined in the behavior of chromosomes. In the *Lilium tigrinum* the intrusion of a third set of homologous chromosomes was a fortuitous and incidental matter. On account of this the mechanism of meiosis which operates for bi-distribution, and the mechanical and physiological activities of microsporogenesis combine to produce the various irregularities which we have herewith described. These operate finally, either singly or in combination, to effect abortion within the cells which comprise the pollen.
Literature Cited


Explanation of Plates

Plates 24 and 25

All figures are concerned with the stages in the microsporogenesis of the diploid and triploid *Lilium tigrinum*.

Fig. 1. Prophase of a P.M.C. of the diploid *Lilium tigrinum* showing synapsed spiremes at pachytene. Iron-haematoxylin. ×1600.

Fig. 2. One pair of homologous chromosomes at pachytene removed from the above and enlarged to show the character and pairing of chromomeres. Matrix shown to be present. ×3340.

Fig. 3. A pair of homologous chromosomes from the diploid *Lilium tigrinum* at diplotene showing not only the dual nature of each chromosome but also their manner of coiling and a true chiasma of chromatids. Iron-haematoxylin. ×3340.
Fig. 4. A trivalent association of homologous chromosomes from the P.M.C. of the triploid *Lilium tigrinum* at pachytene showing the failure of one of the spiremes at certain points to join the other two. Iron-haematoxylin. ×3340.

Fig. 5. Segments of bivalent and univalent spiremes from the triploid at pachytene. Iron-haematoxylin. ×3340.

Fig. 6. A pair of homologous chromosomes from the diploid at diakinensis showing thickening and shortening of chromatids, the lessening of the number of coils, and the terminalization of chiasmata. Aceto-carmine. ×3340.

Fig. 7. A trivalent association of three homologous chromosomes from the triploid at diakinensis showing closer association between two of the chromosomes than between either of these and the third. Chiasmata with false interlocking are present. Aceto-carmine. ×3340.

Fig. 8. A loose association of three homologous chromosomes from the triploid at late diplotene showing irregular coiling of the chromatids and the constriction points all separated from each other. C, constriction. Crystal violet. ×2670.

Fig. 9. A trivalent association of chromosomes at late diakinensis of the triploid showing loose association of the third chromosome and the initial stages in the separation of chromatids in each chromosome. Aceto-carmine. ×1900.

Fig. 10. A univalent chromosome from the triploid at metaphase showing that the parallel chromatids with coiled chromonemata are in the early stages of separating from each other. Aceto-carmine. ×1900.

Fig. 11. a, b, c, d, e, f, g, h, and i are drawings of chromatin elements in the triploid showing all stages in the formation of characteristic microcysts (f, g, h) and their ultimate reduction (i). Lymph vacuoles (L. V.) are present. Crystal violet. ×3340.

Figs. 12, 13, 14, 15, 16, 17. Preliminary stages in the formation of micronuclei in the triploid. Figs. 13 and 14 are of chromosome D. Aceto-carmine. ×3340.

Figs. 18 and 19. Types of chromosome fragmentation by the cell-plates in late anaphase of the triploid. Crystal violet. ×2670.

Fig. 20. Fragments of a chromosome segmented by the cell plate rolling up to form microcysts following meiosis in the triploid. Lymph vacuoles are present. Crystal violet. ×2670.

Figs. 21 and 22. Secondary spindles at the end of the heterotypic division in the triploid showing cell-plate formation leading to the cutting off of microcytes. Crystal violet. ×2930.

Fig. 23. Accessory spindles at the end of the homeotypic division of the triploid showing bifurcation of the phragmoplast resulting in the formation of a microcyte cut from the original daughter cell. Iron-haematoxylin. ×1600.

Plate 26. Photomicrographs

Figs. 24, 25, 26, and 43. Diploid. Figs. 27–42. Triploid.

Fig. 24. Twelve pairs of homologous chromosomes showing bivalent associations at diakinensis. Both terminal and interstitial chiasmata evident. Aceto-carmine. ×487.

Fig. 25. Equal distribution of chromosomes to each pole at heterotypic anaphase. Aceto-carmine. ×230.

Fig. 26. Equal distribution of chromosomes to the poles at the homeotypic division. Aceto-carmine. ×230.

Fig. 27. Thirty-six chromosomes showing two univalents, two bivalents, and ten trivalents, at diakinensis. Aceto-carmine. ×497.

Fig. 28. Heterotypic anaphase with five chromosomes lagging in equatorial plate region. Aceto-carmine. ×234.

Fig. 29. Heterotypic anaphase. No chromosomes lagging in equatorial region. Aceto-carmine. ×234.

Fig. 30. Heterotypic anaphase. Lagging chromosome segmented by cell plate formation. Aceto-carmine. ×234.

Fig. 31. Late anaphase with five chromosomes lagging at time cell plate begins to form. Aceto-carmine. ×234.
Fig. 32. Homeotypic metaphase. In the left daughter cell appear two microcysts* formed from lagging chromosomes of the heterotypic division. Aceto-carmine. ×230.

Fig. 33. Homeotypic anaphase with two lagging chromosomes and microcysts* from the heterotypic division. Aceto-carmine. ×230.

Fig. 34. Heterotypic anaphase in which the chromatids of each chromosome have partially separated. Aceto-carmine. ×607.

Fig. 35. Tetrads with four microspores of average size containing lagging chromosomes from the 2nd division. Microcyte, and microcyst. Aceto-carmine. ×234.

Fig. 36. Tetrad with six microspores and one microcyte. Aceto-carmine. ×234.

Fig. 37. Tetrad showing four microspores and two microcytes, in one of which there is a micronucleus.* Aceto-carmine. ×234.

Fig. 38. Microspores with microcysts. Aceto-carmine. ×154.

Fig. 39. Microspores and microcytes shortly before dehiscence of anthers. Microcysts still present. Ehrlich’s haematoxylin. ×150.

Fig. 40. Mature pollen grains of triploid clone at time of dehiscence showing about 2% germination on artificial medium. Aceto-carmine. ×35.

Fig. 41. Mature pollen grains at time generative cell is formed. Generative cell aborting. Crystal violet. ×150.

Fig. 42. Ripe pollen. Many grains are fully developed and in some the generative cell is formed. Some have grains aborted and are without contents. Heidenhain’s haematoxylin. ×80.

Fig. 43. Germination of pollen, diploid form, on artificial medium. Aceto-carmine. ×35.

* Microcyst, Mst; microcyte, Met; micronucleus, Mn.
Chandler, Porterfield, and Stout: Microsporogenesis in Diploid and Triploid Types of Lilium tigrinum with Special References to Abortions
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