Descriptive and experimental cytology in *Allium*, 1.

The formation of micropollen grains, with some notes on spontaneous chromosome aberrations in *Allium odorum*.*

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Chromosome studies in *Allium odorum* have been reported by Schürhoff (1922), Haberlandt (1923, 1925), Modilewski (1925, 1930), Katayama (1928, 1936), Levan (1931), Ono (1935), Kurita (1950), and others. The author found the extranuclear body which is present in the cytoplasm during microsporogenesis in this species. Hitherto, the extranuclear chromatin found in the cytoplasm of pollen mother cells has been observed usually to degenerate during further successive stages of cell division, while in the species which was the subject of this study it seems probable that the extranuclear body plays a significant rôle as the centre of micropollen grain formation. An account of this study together with a description of spontaneous chromosome aberrations will be given in the present paper.

**Material and methods**

The plants investigated, *Allium odorum* L., were obtained at Nagoya, Gifu and Gujohatiman, the origin or variety name of which being unknown. All the bulbs were tetraploid (2n=32). The observations were based on preparations made by the smear method. The fixatives used were Carnoy’s, Navashin’s, Bouin’s, and La Cour’s (2BE) solution, the staining being acetocarmine, methylgreen-pyronin, and Feulgen’s basic fuchsin.

**Observations**

1. The formation of micropollen grains.

From the onset of meiosis to the formation of mature pollen grains, several extranuclear bodies are observed in the cytoplasm. They appear often as heteropycnotic bodies, being ring-shaped or round, from one to seven in number in each cell. They vary from 2.3 to 5.8 \( \mu \), 3.9 \( \mu \) in average, in diameter. The staining properties of these bodies were ascertained to be Feulgen-positive, and they stained greenish blue with methylgreen dye. Of course, they stain with acetocarmine. In order to ascertain whether or not they contain desoxyribose nucleic acid (DNA), the following test was applied; after the treatment with 0.3 mol trichloracetic acid at 90° C, the bodies

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were stained with methylgreen-pyronin and Feulgen's reaction. The result of these reactions was negative. Thus, doubtless, these bodies contain DNA. They are likely to appear in the extra or supernumerary chromosomes at the meiotic metaphase, but there is no extra fragment in the somatic cells. In the bulbs investigated, these bodies were found without exception. The present author calls them the "extranuclear-bodies", or the "e" bodies in symbolized form.

The frequency of their occurrence in each stage of microsporogenesis is given in Table 1.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of total cells observed</th>
<th>Number of cells having an &quot;e&quot; body or bodies</th>
<th>Percentage</th>
<th>Average percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meiotic prophase I</td>
<td>234</td>
<td>12</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Meiotic metaphase I</td>
<td>217</td>
<td>17</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Meiotic anaphase I</td>
<td>215</td>
<td>17</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>Meiotic metaphase II</td>
<td>189</td>
<td>10</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Meiotic anaphase II</td>
<td>201</td>
<td>14</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Tetrad stage</td>
<td>204</td>
<td>15</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Pollen grain stage</td>
<td>993</td>
<td>35</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>(one-nucleated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen grain stage</td>
<td>974</td>
<td>4</td>
<td>0.4*</td>
<td>(3.7)***</td>
</tr>
<tr>
<td>(two-nucleated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The value, 0.4, is the frequency of the pollen grains having an "e" body or bodies.
** The value, 3.7, is calculated from the frequency of the cells having an "e" body or bodies plus the frequency of the micropollen grains.

Table 2 shows the number of "e" bodies per cell at the stage of one-nucleated pollen grain in which its occurrence is clear cut.

<table>
<thead>
<tr>
<th>Number of &quot;e&quot; bodies</th>
<th>Total number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>&gt;</td>
<td>958</td>
</tr>
</tbody>
</table>

As shown in Table 1, the "e" bodies are found in all the stages. The average frequency of their occurrence up to the tetrad stage is 6.7%. The decrease of the frequency at the stage of two-nucleated pollen grains may be due to the separation of micropollen grains. The process of the micropollen grain formation is shown by photomicrographs. Fig. 1 shows a resting stage in which the pycnotic nucleus is clearly visible, and an arrow "e" indicates the extranuclear body in the cytoplasm.

Almost all the cells observed in this preparation were in diplotene stage. Fig. 2 shows the meiotic prophase, and Figs. 3 and 4 the first metaphase. In Fig. 3 the "e" body is conspicuously large, in Fig. 4 it is smaller. In Fig. 5, the "e" body is situated in the cytoplasm outside the daughter nuclei, which are in the interphase.

In Fig. 6, "e" bodies of different sizes may be observed in cells of the second metaphase.
Fig. 7 is a photomicrograph of the same state. In the cell lying at the right side of the photomicrograph, the small “e” bodies lie in the mother cytoplasm, while another larger one separates from it, probably forming a microcell in a later stage. Fig. 8 shows a normal tetrad. One cell has two “e” bodies. Fig. 11 is a normal one-nucleated pollen grain. Figs. 12 and 13 show pollen grains with one and two “e” bodies respectively. In Fig. 14 a narrow neck is beginning to be formed at the region adjacent to the “e” body; i.e. the formation of a micropollen grain is now in progress by the constriction of the cell wall. Fig. 15 is the polar view of it. Fig. 16 shows a double-pollen grain which consists of one normal grain and one micropollen grain, the latter attached to the former. Fig. 9 is the polar view of a double-pollen grain and normal one. Figs. 17, 18, 19, and 20 show micropollen grains isolated from the mother grains. In most cases their nuclei (“e” bodies) are pycnotic, but in a few cases they are diffuse, as shown in Fig. 20. In addition, it seems probable that the micropollen grain itself begins to grow; i.e. its size increases. Fig. 10 shows pollen grains with the vegetative and the generative nucleus. The differentiation into the vegetative and the generative nuclei is usually delayed in the cells having an “e” body.

Of 974 mature pollen grains examined, 937 were of normal, 4 had an “e” body or bodies, and 33 were of the micropollen grain. Thus, the frequency of occurrence of the two-nucleated pollen grains having an “e” body is actually 3.7%. In Table 1, the value in percentage, 0.4, is the frequency of the pollen grains having an “e” body discounting that of micropollen grains. The percentage of occurrence of the pollen grains having an “e” body in the one-nucleated stage, 3.5, is almost the same as those having an “e” body in the two-nucleated stage, 3.7 (see Table 1).

The micropollen grains become differentiated between the one- and two-nucleated stage of pollen grains by a special mechanism. Generally speaking, the microcell or micronucleus arises as the by-product of an irregular formation of tetrads or from the presence of a stray chromosome. In such cases the formation of the microcell takes place up to the tetrad stage. In the present observations almost all the “e” bodies corresponding to the stray chromosomes were cut off by a wall during the pollen grain formation.

The meiosis of *A. odorum* takes place under high temperature conditions in summer (outdoor maximum temperature about 37°C in Japan).

It is the next subject to be studied whether such an environmental condition is related to the occurrence of the “e” body. The flower buds were placed in a low temperature chamber of 5-7°C for seven days. The meiosis proceeded slowly under this environment. The cells having an “e” body or bodies were still present in the plants under observations.

The micropollen grains were, however, differentiated no further. On the artificial medium (10% saccharose and 2% agar), both the pollen grains with or without an “e” body permitted germination, while the empty grains and the micropollen grains did not. Often, the “e” body could not be distinguished from free fragments or ring chromosomes due to the chromosome breakage and reunion (see Figs. 20, 22, 23, 29,
Figs. 1-23. The ex ranuclear chromatin ("e" body) found in the cytoplasm is indicated by the arrow "e". 1, resting stage. 2, first prophase. 3-4, first metaphase. 5, interphase. 6-7, second metaphase and prophase. 8, tetrad. 9, isolation of a micropollen grain (polar view). 10, mature pollen grains. 11, normal grain. 12-13, pollen grains having one and two "e" bodies respectively. 14-15, The narrow neck begins to be formed at the region adjacent to the "e" body. 16, a micropollen grain formed by discharge of the "e" body (side view). 17-20, micropollen grains of different size. 21-23, ring-shaped "e" bodies and abnormal division (bridge and fragments).
Figs. 24-38. Chromosome aberrations in PMCs. 24, fragmentation and fusion. 25, double nucleus and extraordinary fragmentation. 26, laggards. 27-31, fragmentations. 32, irregular anaphase. 33, fragments, micronuclei and double nucleus in telophase I. 34, micronuclei in telophase I. 35-38, abnormal tetrads.
and 30). In plants under low temperature conditions, it was observed that chromosome breakage was very infrequent as compared with plants in their natural environment. However, there is no significant difference between the frequency of occurrence of the cells having an “e” body in treated and untreated ones. Thus the occurrence of the “e” body seems to be independent of chromosome aberrations.

2. Chromosome aberrations.

Meiotic abnormalities were observed only in the plants obtained at Nagoya. The proportions of abnormal cells varied with different flower buds, sometimes even with different anthers in a bud. The synchronization or non-synchronization of the division phases within each anther relates closely to the kind of abnormalities, as will be described later.

Chromosome pairings of various types were observed during the first metaphase. The configuration $8_{14}, 32_1$, and several intermediate combinations were detected.

The following types of irregular divisions were frequently found in the first anaphase and telophase; the lagging chromosome caused by non-pairing in the first metaphase (Fig. 26), the single bridge without fragments due to the failure of the end gene to divide (Fig. 21), acentric fragments (Figs. 21, 22, 23, 27 etc.), a chromatid bridge accompanied by a fragment (Fig. 24), the multinucleated cell (Fig. 33), anomalous anaphase (Fig. 32), misdivision of the kinetochore, and disturbance of the spindle bipolarity.

Sometimes the breakage was so extreme that an accurate analysis was difficult (e.g. Fig. 25). Such divisions give rise to very irregular pollen tetrads, as shown in Figs. 35, 36, 37, and 38, and to the telophases with micronuclei, as shown in Figs. 33 and 34. The chromosome breakages were of the $B^H$ (chromosome unit) type.

Several types of meiotic irregularities occurred which may be associated with the disturbed degree of synchronization of the division phases within each anther, and this relation was analyzed in the first anaphase. This result is given in Table 3.

Often, the anthers investigated had a pollen-sac which contained conspicuously non-synchronized pollen mother cells. The percentage of occurrence of various meiotic stages found in six anthers belonging to a flower bud is, for example, shown below.

<table>
<thead>
<tr>
<th>Anther 1</th>
<th>Telophase [100%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anther 2</td>
<td>Prophase [2.8%], Metaphase [60.3%], Anaphase [28.0%], Metaphase [25.3%], Anaphase [5.3%], Tetrads [1.1%]</td>
</tr>
<tr>
<td>Anther 3</td>
<td>Anaphase [100%]</td>
</tr>
<tr>
<td>Anther 4</td>
<td>Prophase [1.8%], Tetrads [98.2%]</td>
</tr>
<tr>
<td>Anther 5</td>
<td>Tetrads [100%]</td>
</tr>
<tr>
<td>Anther 6</td>
<td>Prophase [2.4%], Metaphase [3.9%], Anaphase [4.1%], Metaphase [3.1%], Anaphase [28.6%], Tetrads [57.9%]</td>
</tr>
</tbody>
</table>

From Table 3, it was concluded that there was a positive correlation between the normality of division phases (normal synchronization) and the occurrence of chromosome breakage within one and the same anther, and there was a parallel relation between the non-synchronization of division phases and the occurrence of other irregularities (mainly, lagging chromosomes).
Discussion

The extranuclear chromatin in the cytoplasm has hitherto been described in many instances, but it is not apparent whether the "extranuclear chromatin" described in each instance was really chromatin or of nuclear origin. More recently, Sparrow and Hammond (1947), Darlington and La Cour (1946), and Cooper (1952) reported that Feulgen-positive bodies present in the cytoplasm at the onset of meiosis are to be found in many species of plants. These bodies vary in size 0.3 to 7 μ; their number per cell varies from one to sixty. The Feulgen-positive body in the cytoplasm has been interpreted (1) as being caused by the transfer of DNA from nucleus to cytoplasm (Sparrow and Hammond's interpretation), (2) as due to the transfer of DNA from tapetum to the microsporocytes (Cooper's interpretation).

The bodies found in the present species are different from those of plants mentioned above in the following respects. The bodies are 3.9 μ in average diameter, and their number per cell varies from one to seven. Their shape is round or ring-like. They exist through all the division cycles. The "e" body plays an significant rôle as a centre of the micropollen grain formation. The presence of this "e" body, which causes the rise of micropollen grains, seems to act as an obstacle to the normal pollen grain formation. The "e" body does not divide nor fuse, but its presence continues through the microsporogenesis. In a few cases it transforms into the micronucleus, or the nucleus of a microcell. The plants obtained from Gifu and Gujohatiman were normal meiosis, but the cells having an "e" body or bodies were often found. This fact shows that it is not caused by meiotic abnormalities. In the plants obta-
ined from Nagoya meiosis is very abnormal, and also cells having an "e" body or bodies are frequently found. Thus the possibility that they are the result of meiotic abnormalities is not eliminated. In the flower buds treated by low temperature—the material obtained from Nagoya—the chromosome aberrations were few, while the frequency of occurrence of cells having an "e" body was almost constant. From the evidence presented above, it may be concluded that the extranuclear chromatin in the cytoplasm is not due to abnormal meiosis. Its occurrence is presumably caused by a migration of chromatic substances from nucleus to cytoplasm at pre-meiotic mitosis. In the living state of pollen grains the "e" body is also visible under the phasecontrast microscope; therefore there is no possibility of its being an artifact.

The problem whether or not the male cell is accompanied by "e" bodies at the time of fertilization is left undecided.

The kinds of chromosome aberrations are quite similar to those induced by X-ray, high or low temperature, chemicals, genetic control, aging, and other causes. Fukasawa (1952) has described the abnormal meiosis induced in winter by vernalization and long-day treatment in Triticum and Aegilops. He observed the relation between the different stages of pollen mother cells in the same pollen sac and failure of the pairing at the first metaphase but his study was concerned only with the problem of non-pairing. Thus the relation between the synchronization of division phases and chromosome breakage was left untouched. Rees (1952), who studied asynapsis in conjunction with spontaneous chromosome breakage in the pollen mother cells of Scilla sibirica, concluded that "Asynapsis and chromosome breakage are interpreted as being independent consequences of a common cell disturbance with a sharp threshold differentiating genetically equivalent pre-meiotic cells". In fact, Mather (1934) has shown that the fragmentation does not prevent the formation of chiasmata. From this point of view, the findings of the present author are coincident with those of Rees. The fact that other irregularities except for chromosome breakage, mainly lagging chromosome, appear very frequently in anthers containing non-synchronized pollen mother cells, and that chromosome breakage is found very often in the anthers containing synchronized pollen mother cells may be shown to be caused by independent mechanisms.

Summary

1. Feulgen-positive bodies were found in the cytoplasm through all the stages of microsporogenesis in Allium odorum.

2. The extranuclear bodies ("e" bodies) mentioned above are round or ring-shaped, and their number within a single cell varies from one to seven. The average diameter is about 3.9 μ. The average frequency of occurrence of cells having an "e" body or bodies in all stages, discounting the stages of one- and two-nucleated pollen grains, is 6.7%.

3. During the development of pollen grains the "e" bodies exist as the centre of micropollen grains isolated from the mother cell.

4. The occurrence of the "e" bodies is independent of meiotic irregularities.

5. The frequency and kinds of chromosome aberrations in this species were observed. In the anthers which contain synchronized and non-synchronized pollen mother
cells of the division phases, the frequency of occurrence of the cells accompanied
by chromosome breakage is an average of 5.3% and 1.1% respectively, while that of
other irregularities (mainly, lagging chromosome) is an average of 0.7% and 7.9%
respectively. From these results, it is concluded that chromosome breakage and
non-pairing seem to result respectively from separate mechanisms, independent of each
other.

In conclusion the author wishes to express his sincere thanks to Professor T.
Shimamura for his kind criticism and valuable advice. Thanks also due to Dr. M.
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Literature cited

Cooper, D. C. 1952. The transfer of desoxyribose nucleic acid from the tapetum to the microspo-
156, 875-876.
Fukasawa, H. 1952. Some notes on the abnormal meiosis of wheats matured in winter (Japanese
43, 559-564.
431-441.
Kurita, M. 1950. Uber die Chromosomen von Allium odorum L. Mem. Ehime Univ., Section II,
1, 37-42.
Sparrow, A. H. and M. R. Hammond. 1947. Cytological evidence for the transfer of
desoxyribose nucleic acid from nucleus to cytoplasm in certain plant cells. Amer. Jour. Bot.,
34, 439-445.