CYTOLOGICAL OBSERVATIONS ON A *SALVIA*-HYBRID

by

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(With plates I-III)

Introduction

While making collections of cytological materials from *Salvia Jurišičii* Koš. an aberrant specimen was noticed among many other specimens of *Jurišičii*. This specimen markedly differed from the normal species: it was stronger in growth and different in foliage and it was characterized by complete sterility as regards the pollen and fruit production. After flowering all the flowers shrivelled and fell. It was something new and at the first sight was suspected of being a hybrid which had arisen from an accidental natural cross between *Salvia Jurišičii* Koš. and some other species of *Salvia*.

In order to understand the nature and origin of this hybrid, a cytological study of the developing pollen mother cells was undertaken. The necessary cytological materials were taken from a plant now under cultivation in the University Botanical Garden, Beograd, where this hybrid had spontaneously appeared. As a fixing fluid Carnoy's solution with chloroform (6:2:2) was employed. Although perfect fixation was difficult to obtain, the results of fixation, however, may be regarded as fairly satisfactory. The materials were treated by the paraffin method in the usual way. The sections were cut to a thickness of 8—10 μ and stained exclusively with Heidenhain's iron-alum haematoxylin.

The collections of cytological materials were made chiefly in June 1927 on a bright warm day between 10 and 11 a. m. In order to avoid the possible objection that some direct injury was given to the anthers in handling the living materials, the whole young inflorescences were cut with a sharp razor and immediately dropped into the fixing fluid. At the same time the young inflorescences of *Salvia Jurišićii* Koš. were gathered and fixed in Carnoy's solution. The collecting of materials from both normal and aberrant plants grown in nearly identical environmental conditions and the treating of the materials with the same cytological technique made it possible to compare the cytological data and to give an adequate explanation of the nuclear phenomena observed.

In the course of my study of the cytology of both *Salvia Jurišićii* Koš. and *Salvia*-hybrid two publications have appeared, namely: Scheel's „Karyologische Untersuchung der Gattung Salvia“ (1931) and Uittien's „Salvia-Bastaarden III“ (1933). In Scheel's paper the haploid number of chromosomes in *Salvia Jurišićii* Koš. was established as 11. My investigations only go to confirm the exactness of this number, as shown in our Fig. 10. Uittien has discovered a *Salvia*-hybrid originating from a cross between *Salvia Jurišićii* Koš. and *Salvia nemorosa* L. and given a full description of the hybrid plant. Following his description it appears very probable that our *Salvia*-hybrid might also be an offspring of the same parentage.

In the following account I propose to describe some of the cytological phenomena, the most significant of which are the process of nuclear extrusion (cytomyxis) during the prophase of the first meiotic division and the behaviour of the chromosomes through both meiotic divisions.

Cytomyxis

The condition known since Gates's investigations on *Oenothera gigas* and *Oenothera biennis* (1911) as cytomyxis, by which nuclear substances are ejected from one pollen mother cell into the cytoplasm of the adjacent one, was frequently found to occur in the pollen mother cells of our *Salvia*-hybrid. The following is a brief description of this process.

The development of the sporogenous tissue seems to be carried out in a regular manner and the archesporial cells are quite normal in appearance. When the nuclei of these cells enter upon the prophase of the heterotypic division, the cytomyctic phenomena are observed to take place in the majority of mother cells examined. Our Figs. 1—5 simply display representative stages in the behaviour of the nuclei during the various stages of cytomyxis.

The very beginning of the cytomyctic process is shown in Fig. 1. The nucleus of the pollen mother cell to the right has taken an excentric position and is in contact with the cell wall which separates both neighbouring mother cells. The nucleolus has also moved to the same side, whereas the beaded chromatin threads are distributed throughout the greater part of the nuclear cavity. Evidently fhe nucleus is just coming out of the synizetic stage. A portion of the nuclear substances has already penetrated the cell walls and entered the cytoplasm of the adjoining mother cell. The extrusion has probably taken place through the openings in the cell walls, following the path of the cytoplasmic connexions (plasmodesms) which unite the protoplasts of two neighbouring cells. Two very fine connexions joining the extruded material to the parent nucleus are conspicuous, owing to the increase of their staining power. Arrived in the cytoplasm of the adjacent cell, the extruded nuclear material becomes surrounded by a clear space filled up with the extruded karyolymph of the parent nucleus. In contact with the cytoplasm of the invaded cell a definite nuclear membrane is formed, and a few chromatin granules, pressed up against the new nuclear membrane, may be seen.

Fig. 2 shows the cytomyctic process at a very advanced stage. From the nucleus in the left cell only one small darkly stained globule is to be seen, still retaining its connexion with the extruded material by means of a very thin and distinct filament. The nucleolus has also taken part in extrusion. In the cytoplasm of the invaded cell there are now two nuclei with their definite nuclear membranes pressed up against each other. Though the nuclear substances were forced to pass through very narrow openings in the cell walls, the structure of the extruded nucleus resumed an apperance which closely ressembles that of the original nucleus. A similar process of extrusion is beginning to take place from the nucleus of the invaded cell into the cytoplasm of the next cell to the right, and already one small globule has entered the cytoplasm of the latter (Fig. 2).

A similar row of nuclear migrations was very often observed in our preparations. But these migrations do not necessarily occur always in one and the same direction and are not exhibited by all the pollen mother cells of a loculus. Very often cases were encountered, in which the nuclear material was ejected from one mother cell into two neighbouring ones, as shown in Fig. 3.

Figs. 4 and 5 show the extrusion of nuclear material during the late stages of diakinesis. Cases were found, in which the extruded substance had lost its connexion with the original nuclear mass. Under such circumstances one minor accessory nucleus was frequently seen in addition to the major nucleus of the invaded cell (Fig. 4). The dwarf nuclei contained always a certain, but usually a very small number of extruded chromosomes. That the minor nuclei are derived from the nuclei of the neighbouring cells simply by the process of cytomyxis, our Fig. 5 amply demonstrates.

Nuclear migration in later stages than diakinesis was quite absent from our preparations. Also no extrusion was found to occur from the pollen mother cells into the adjoining cells of the tapetum.

As to the subsequent history and fate of both the invaded and enucleated cells, I must here remark that no case was observed in which these cells were capable of continuing through further stages of meiosis. They invariably showed, sooner or later after the extrusion had taken place, evident signs of an approaching disintegration. Later they were encountered in preparations as amorphous deeply stained masses in which nothing of the previous nuclear structure could be distinguished.

Behaviour of the tapetum

The tapetal cells form a single layer surrounding the sporogenous tissue at an early stage of the anther development. At about the time the nuclei of the pollen mother cells enter upon the synizetic stage, the nucleus in the tapetal cells divides mitotically. After the binucleate stage has been attained, the tapetal cells in some anthers remain in a more or less inactive state, thus foreshadowing their impending disintegration. One very interesting fact should be noted: in those anthers showing tapetal cells in an anomalous condition the cytomyctic phenomena were found to be of general occurrence.

Behaviour of the chromosomes

Considerable attention was given to the shape and size of the individual chromosomes as they appear at diakinesis, in order to identify them and thus obtain their exact number and understand their method and degree of pairing.

The chromosomes are not all paired at diakinesis. The number of paired chromosomes frequently observed was 8. Eight *Jurišićii* chromosomes mate with eight of the second parent to form bivalents, leaving 3 chromosomes as univalents. Figs. 6 and 7 represent the nuclei at diakinesis with 8 bivalent and 3 univalent chromosomes, the bivalents being mostly attached end-to-end. It is interesting to note that the bivalents strikingly ressemble in appearance those of *Salvia horminum*, as shown in Scheel's Fig. 31.

Occasionally one trivalent chromosome was plainly visible, in which case one bivalent was probably accompanied by one of the univalents.

In the early stages of diakinesis the fibres connecting the members of the gemini are distinguishable. These gradually contract, so that both homologous finally are closely united to each other. Chromosome condensation does not proceed synchronously among all the chromosomes, so that it is not unusual to find some pairs already well condensed, while others show a thread-like appearance in a more or less high degree.

During diakinesis a certain number of chromosomes were seen to be closely connected with the nucleolus by a fine and elongated chromatic filament (Figs. 8 and 9). These are satellite chromosomes which are contacted with the nucleolus by their satellites. The satellite chromosomes are clearly distinguishable as darkly stained granules or caps on the surface of the nucleolus. Fig. 9 shows two fibres connecting the satellites with their corresponding chromosomes, while of the third chromosome only the satellites appears to be left. A similar relation of the satellites to the nucleolus has been reported by several investigators in the case of a great number of plants. Recently Smith (1933) has given attention to these structures in his study on *Galtonia candicans*, where also the necessary literature is quoted.

Owing to the presence of both types of chromosomes (paired and unpaired) at diakinesis, a varying distribution of the univalents to the daughter nuclei was expected to occur during the heterotypic division. And this was indeed shown in a large number of counts made in favourable interkinesis nuclei. The numbers of the chromosomes, before they had lost their morphological individuality, could be ascertained to be usually 10 or 11, as shown in Fig. 13. But the number may be sometimes as low as 8. Such a low chromosome number was detected at the homoiotypic anaphase of the under diad of the megaspore mother cell. Fig. 11 clearly demonstrates it. Unfortunately the upper diad was missing in the preparation and therefore it is not known whether in its nucleus are included all the 3 univalents in addition to 8 homologous of the bivalents.

A study of the heterotypic anaphase showed a lagging of the chromosomes and an irregular arrangement on the spindle (Fig. 12). A large number of the extranuclear granules, taking the chromatin stain, were seen to be scattered about in the surrounding cytoplasm. The condition shown in Fig. 12 is common. Many cases were observed, in which no chromosomes could be detected on the spindle, whereas on the other hand a much increased amount of the cytoplasmic chromatin was present, and the spindle fibres as well as the surrounding cytoplasm assumed a more or less muddy appearance. In addition, the spindles fibres were greatly thickened. All these facts indicate that an ejection of chromatin from the spindle, accompanied with the dissolution of karyoplasm (chromatolysis), had taken place. Such an excessive loss of chromatin is evidently a pathological condition, which is due to disturbances consequent upon hybridization.

The homoiotypic division was frequently observed. The spindles were found to lie either parallel or at angles to one another. During the anaphase and telophase an increasing amount of the cytoplasmic chromatin was present in a marked degree (Fig. 14).

As a result of the second meiotic division four tetrad nuclei were formed, which were unequal in size and very poor in chromatin substance. The numbers of the chromosomes incorporated with the tetrad nuclei were extremely varying. Fig. 15 shows two tetrads, each of them containing 3 nuclei (the fourth being cut off from the section), which are unequal in size and in which varying numbers of chromatic granules are visible. In the surrounding cytoplasm a large number of deeply stained granules are present.

Under such conditions the nuclei of the microspores are, as it was expected, not able to carry on their function in fertilization. The microspores which arose from these irregular and abnormal meiotic processes were not able to separate from one another. Hence our *Salvia*-hybrid produces no functional pollen grains.

Concluding remarks

From the foregoing account we may say that our *Salvia*-hybrid is characterized by all those conditions which are recognized to be characteristic of many well known hybrids, such as heterosis, pathological condition in the tapetum, irregular meiosis, occurrence of cytoplasmic chromatin, cytomyxis, failure of the tetrads to separate from each other, pathological condition in the cytoplasm, sterile pollen grains.

The pollen mother cells showed evident signs of degeneration at an early stage of meiosis. This degeneration was preceded by feeble condition of the tapetum and consequently by the occurrence of the cytomyctic phenomena. According to Shoji and Nakamura (1928) the early pathological condition of the tapetum in *Asparagus officinalis* is considered to be „one of the factors which bring out the degeneration of pollen-mother-cells" (p. 146). A similar comment is made by Whyte (1929) in his study on the interspecific hybrids of *Nolana*. He stated that if „the tapetum began to disintegrate, cytomyxis would commence in the sporogenous tissue, and general anther degeneration would set in" (p. 114). This is in accord with the findings in our *Salvia*-hybrid. The cytological examination brought out the fact that the degeneration of the tapetal cells was accompanied by cytomyctic phenomena in the pollen mother cells and consequently by their degeneration.

Various views have been put forward to explain the nature and origin of the cytomyctic phenomena, but no agreement has as yet been reached. Because of the divergence of opinions, I have thought it of interest to give some attention to these peculiar cytological phenomena.

The extrusion of the nuclear material from one pollen mother cell into the cytoplasm of an adjoining one was first observed by Koernicke (1902) in the pollen mother cells of *Crocus vernus*. Since then the same process has frequently been noted, figured and described by many investigators, such as Gregory (1905) in a sterile race hybrid of *Lathyrus odoratus*, Digby (1909, 1910, 1912, 1914) in *Galtonia candicans*, *Primula*, *Crepis*, Rosenberg (1907, 1909) in *Hieracium*, *Drosera*, *Crepis*, Gates (1911, 1927) in *Oenothera gigas*, *Oe. biennis*, *Lathraea*, Nakao (1911) in *Secale*, Fraser (1914) in *Vicia faba*, West and Lechmere (1915) in *Lilium candidum*, Sakamura (1916, 1920) in *Vicia faba*, Farr (1918) in *Magnolia*, Tischler (1921) in *Phragmites*, Yasui (1921) in *Papaver* hybryds, Gates and Rees (1921) in *Lactuca sativa*, Sinotô (1922) in *Iris japonica*, Longley (1924) in *Crataegus*, Hurst (1925) in *Rosa*, Roscoe (1927) in *Wisteria*, *Typha*, Ruttle (1927) in *Nicotiana*, Hicks (1928, 1929) in *Scirpus*, *Eleocharis*, Erlanson (1929) in *Rosa*, Inaryama (1929) in *Iris Kaempferi*, Whyte (1929) in *Nolana* hybrids, Woodworth (1929, 1930, 1931) in *Betula*, *Corylus*, *Alnus*, *Juglans notha*, *Pterocarya Rehderiana*, Church (1929) in *Gramineae*, Scheel (1931) in *Salvia silvestris*, Youngman (1931) in *Thespesia populnea*, Stebbins (1932) in *Antennaria*, Kattermann (1933) in intergeneric hybrids of *Triticum*.

According to one of the views the cytomyxis is considered to be merely an artefact produced either by unsatisfactory and faulty fixation (Rosenberg, Sinotô), or by unsufficient care in handling the living material (by pressure, Woodworth, 1931), or by abnormal variation in temperature (Inaryama). Another view which is opposed to the above mentioned is that the cytomyctic phenomena represent a continuous process in meiosis (Gates, Fraser, Digby) and it has even been suggested that they are to be regarded as a normal phase in meiosis (West and Lechmere).

Several other investigators suggested another explantion. Thus Erlanson believes the cytomyctic process „to be due partly to the imperfect fixation and partly to disturbances consequent upon hybridization" (p. 499). Kattermann is of opinion that the cytomyxis may be produced „sowohl unter natürlichen abnormen Bedingungen wie auch unter künstlich geschaffenen abnormen Verhältnissen" (p. 780). According to Church (1929) the cytomyxis cannot be simply explained as an artefact and this abnormality is thought to be in connection with the hybrids. He concludes as follows: „Finally, even if the phenomenon can be proved not to be an exclusively hybrid characteristic, its occurrence is manifestly increased in obvious hybrids such as are described in *Panicum*" (p. 77). Woodworth in his study of the microsporogenesis in *Pterocarya Rehderiana* (= *P. fraxinifolia* × *P. stenoptera*) came to the conclusion: „If this cytomyxis is due to the instability of a heterozygous protoplasm, this is then the ultimate cause of the cytomyxis rather than the fixative" (p. 867). Stebbins found that the cytomyxis occurred more frequently in the hybrid and parthenogenetic species of *Antennaria* than in the normal sexual forms, and concluded that whether this phenomenon is „an artifact or not, it reflects in these cases an abnormal state of the cytoplasm, which may often be due to a hybrid condition“ (p. 336).

I have never observed the cytomyctic phenomena in the pollen mother cells of *Salvia Jurišičii* Koš., though materials of it were collected at the same hour and treated with the same cytological technique as materials of our *Salvia*-hybrid. Therefore I am strengthened in the belief that the heterozygous protoplasm may be responsible for both the feeble condition in the tapetum and cytomyctic phenomena.

Another feature of the hybridity exhibited by our *Salvia*-hybrid is the appearance of the chromatin granules in the surrounding cytoplasm during both meiotic divisions. These structures have been reported by many investigators to appear in the cytoplasm of a great number of species of plants which are of hybridogenous origin. The following works may be cited: Juel (1900) in *Syringa rothamagensis*, Rosenberg (1904) in *Drosera* hybrid, Tischler (1906, 1910) in *Bryonia* and banana, Farmer and Digby (1910) in hybrid ferns, Kuwada (1911) in *Zea*, Carruthers (1911) in *Helvella crispa*, Osawa (1913) and Sears (1917) in *Taraxacum*, Yasui (1921) in *Papaver*, Blackburn and Harrison (1921) in *Rosa* hybrids, Campin (1924) in *Solandra*, Tokugawa and Kuwada (1924) in *Canna*, Longley (1924) in *Rubus*, *Crataegus*, Bleier (1925) in *Trifolium*, Tschermak and Bleier (1926) in *Aegilotricum*, Roscoe (1927) in *Wisteria*, Fisk (1927) in *Zea*, Hicks (1927) in *Cyperaceae*, Church (1929) in *Gramineae*, Woodworth (1929, 1930) in *Corylus*, *Alnus*, *Juglans*, *Pterocarya*, Longley and Sando (1930) in intergeneric hybrids of *Triticum*, *Secale*, *Aegilops*, Michaelis (1930) in *Oenothera*, Graustein (1930) in *Selaginella*, Stebbins (1932) in *Antennaria*, Beck and Horton (1932) in *Bromus*.

As to the origin and meaning of the cytoplasmic chromatin, various interpretations have been proposed. The majority of investigators believe their probable origin to be in the extrusion of chromosomes (elimination of unpaired chromosomes and laggards). Bleier noted the deeply stained bodies in the cypoplasm factors, such as moisture, light, temperature, chemical agents, mechanical influences, etc. All these factors may affect the developing sporocytes during the critical period of the formation of the spores, and so the latent disharmony in the heterozygous protoplasm may become manifest. In the hybridity itself lies the chief source of all those abnormalities and pathological conditions which interfere with the anther developmet and completely destroy the fertility. The hybrids being governed by a latent disharmony are very sensitive to environmental changes. All the abnormalities in meiosis of the hybrid plants may well be understood and explained in the light of Tischler's interpretations. This eminent cytologist stated: „Die Sterilität ist dadurch bedingt. dass zwei Sexualzellen zusammentreten sind, die eine nicht identische Entwicklungs*richtung* und *-tendenz* besitzen.... Beim Eintritt des Individuums in den besonders „kritischen" Zeitpunkt der generativen Phase wird sich dann die starke „Harmoniestörung" auch äusserlich dokumentieren" (1928, p. 144). In another, recently published article Tischler (1930) has pointed out that for hybrids „eine übergrosse Labilität des Zellgeschehens in der kritischen Periode der Reifeteilungen charakteristisch ist" (p. 153).

In my opinion all the abnormalities in meiosis may be considered as evidence of the hybridity and impurity of species. Even in those species which are recognized to breed pure, the slightest occurrence of the cytomyxis, irregular distribution of chromosomes, unsufficient pairing, cytoplasmic chromatin and other abnormalities in meiosis, might be indicatives of an actual or remote hybrid origin. Theses abnormalities, though they do not interfere with the fertility, may be understood as reminiscence of the hybridization which had taken place in the past. The process of stabilization in genome is not yet come to the end. On the other hand the slight occurrence of the abnormalities in meiosis in „pure" species might be explained as a sign of the disturbances in genome which are in statu nascendi and which in the course of time may result either in the loss of sexuality or in the extinction of the present form or in the production of new forms (mutants).

The present article leaves the question of the second parent of our *Salvia*-hybrid open. It is not impossible that this hybrid may be identical with the hybrid *Salvia Jurišićii* Koš. × *Salvia nemorosa* L. which has been described in Uittien's paper on

„Salvia-Bastaarden“. Unfortunately *Salvia nemorosa* has not as yet been examined from the karyological point of view, so that the number of its chromosomes and their behaviour during meiosis is unknown. A karyological study of this species has already been undertaken by the writer of the present article and the results will be soon published.

Summary

1) Some abnormalities during meiosis were observed in a *Salvia*-hybrid, the most significant of which are nuclear extrusion (cytomyxis), occurrence of univalents and laggards, appearance of cytoplasmic chromatin.

2) Our *Salvia*-hybrid is characterized by complete sterility as regards the pollen and fruit production. This indicates that the protoplasms of the parents differ in a large degree as regards their hereditary tendencies, and that the parents are not closely related.

3) A feeble condition of the tapetum was observed to precede the cytomyctic phenomena. Cytomyxis was found to take place only during the prophase of the heterotypic division. Both the invaded and enucleated pollen mother cells sooner or later disintegrate.

4) Both types of the chromosomes are present in diakinesis: 8 paired and 3 unpaired. The distribution of the chromosomes is acomplished according to *Drosera*-scheme.

5) During both meiotic divisions a large amount of cytoplasmic chromatin was observed. These darkly stained bodies are thought to be of chromosomal origin. Cases were observed in which no chromosomes were on the spindle fibres, all the chromatin being ejected and scattered in the cytoplasm. The muddy appearance of the spindle fibres and cytoplasm may be explained by the dissolution of the chromatic substances (chromatolysis).

6) It is thought, in agreement with Tischler's interpretations, that the hybrid protoplasm is very sensitive to the changes of environmental factors, owing to the lack of harmony between the two different hereditary potencies of the parents. The hybridity may be responsible far all the abnormalities which occur in the critical period of meiotic divisions.

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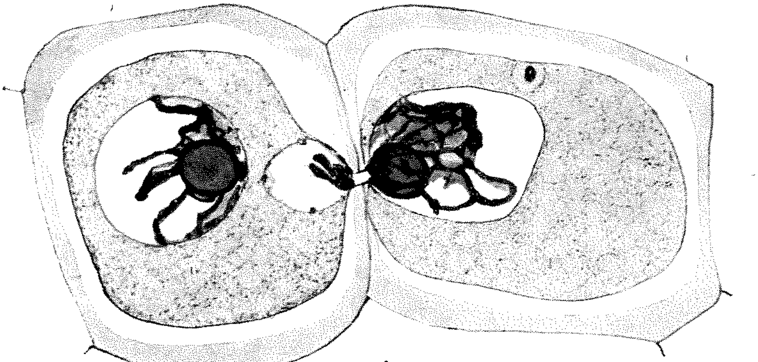

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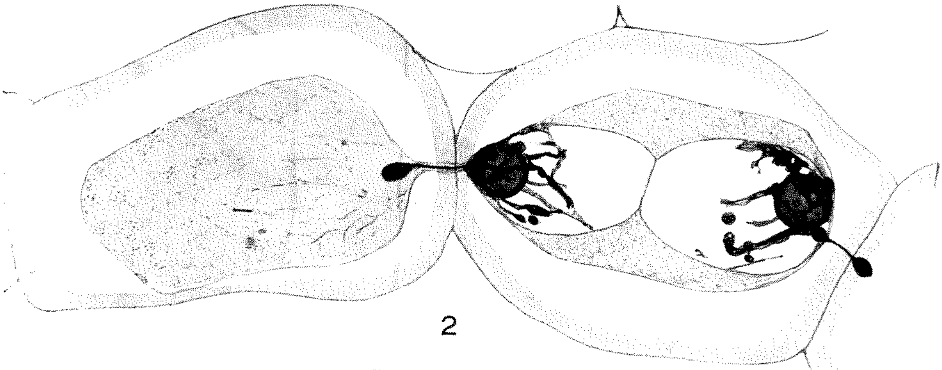

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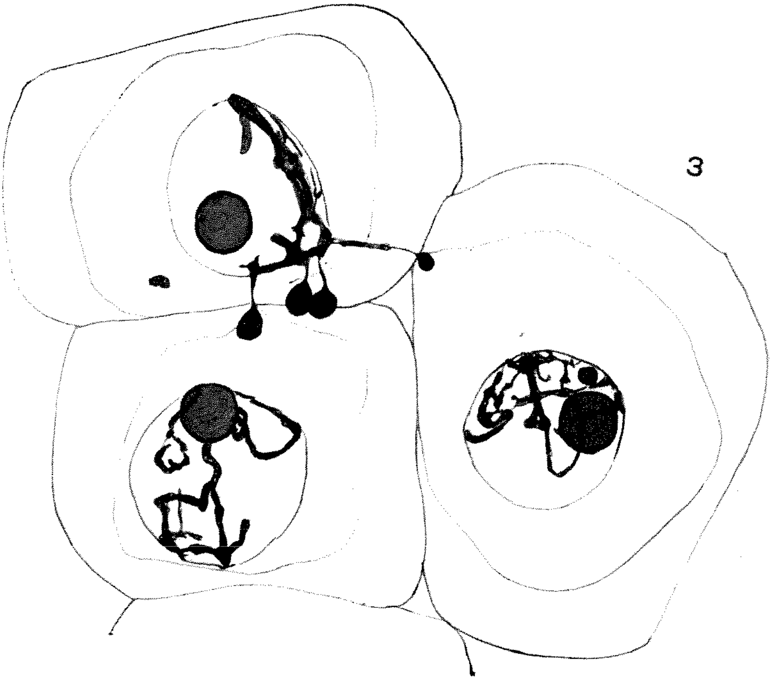

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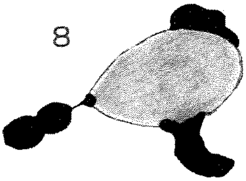

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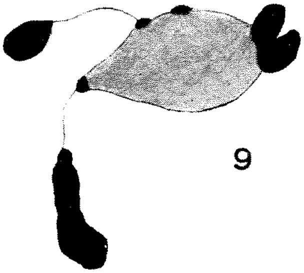

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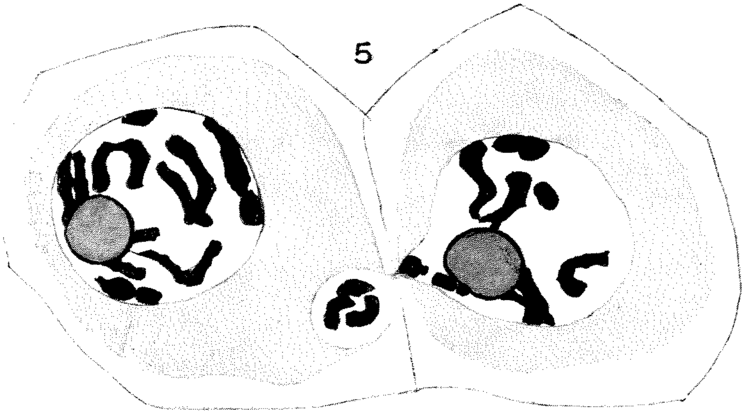

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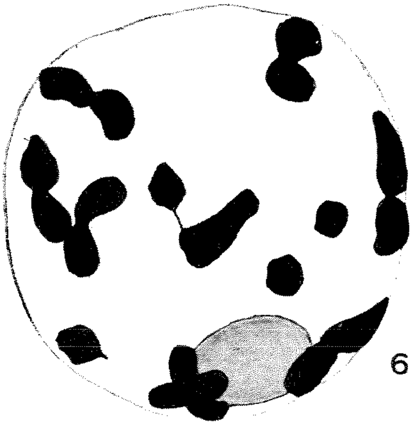

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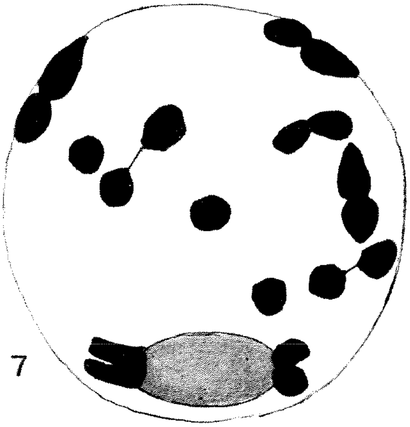

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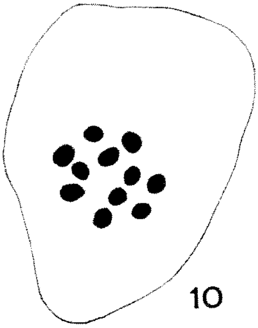
Bull. Inst. et Jard. Bot. Univ. Beograd. Tome II **Pl. III.**

Lj. M. Glišić: **Cytological observations on a Salvia-hybrid**



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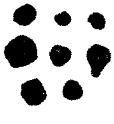

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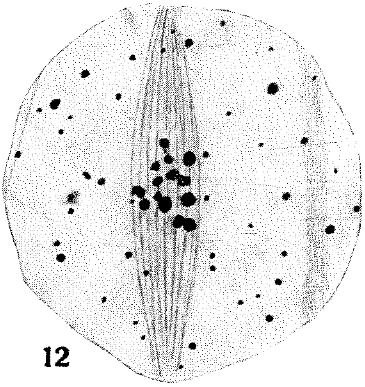

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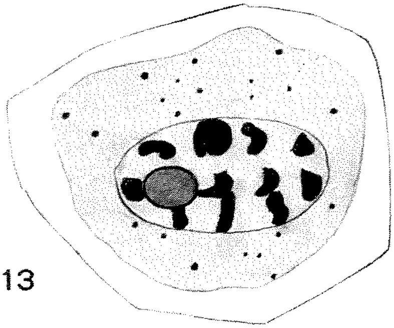

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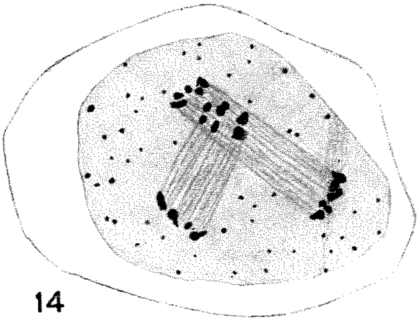

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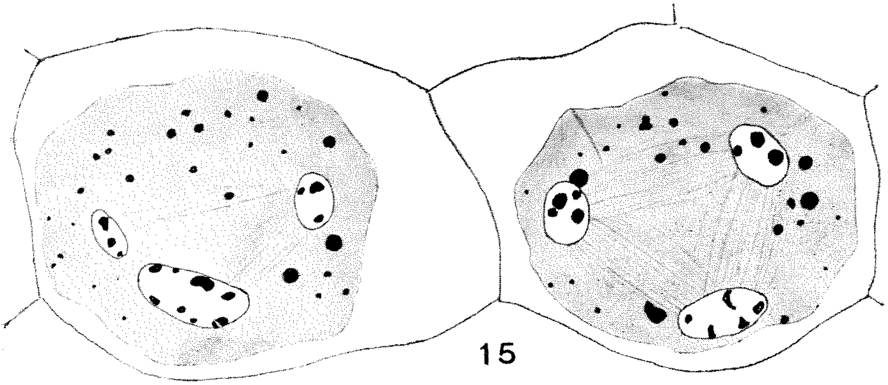

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