MACROSPOROGENESIS AND DEVELOPMENT OF THE MACROGAMETOPHYTE OF DATURA TATULA L.

by

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Introduction

During a preliminary examination of prepared microtome sections made through the ovaries of *Datura tatula* L., collected late in the fall, some unusual features in the development of the macrospores and macrogametophyte have been noticed. This latter structure appeared to be mono-, di- or tetrasporic in origin. It was thought, at first, that these abnormalities might be due to the influence of low temperature, to which the plants, grown in te field, were subjected during cold atumm days. But since the plants used in this investigation were grown under almost variable external conditions as far as temperature and other environmental factors are concerned, no causal relation could be maintained. There is obviously need for experimental work to ascertain whether and inasmuch the environmental stimuli might be responsible for the abnormalities observed, and to determine the importance of the various factors when acting alone.

When one compares conceptions concerning the taxonomy of *Datura tatula* L. and its closest affinities to the related form *Datura stramonium* L., different statements are encountered. While some investigators regard them as being of separate specific rank, as recognized by Linnaeus, who described and named both species, the other botanists hold that *Datura stramonium* L. and *Datura tatula* L. do not exibit sufficient differences to warrant their treatment as distinct and independent species. They look upon *Datura tatula* L. as a race or variety of *Datura stramonium* L. (*Datura stramonium* β *chalybaea* Koch, *Datura stramonium* β *tatula* (L.) Dun.). According to Bitter (1903) the *stramonium*- and *tatula*-forms are to be considered as parallel types, differing in the coloration of their petals. A condition, which was also reported for a number of the solanaceous and other plants, e. g. *Nicandra physaloides* with green (*viridis*-form) and violet flowers (*violacea*-form), *Cichorium intybus* (blue and white), *Salvia Jurišićii**) Koš. (violet and white), etc.

What significance may be attributed to this pair of characters (violet-green, violet-white) and are they accompanied by any visible alterations in genom, which would permit to distinguish both taxonomically related forms? Whether the abnormalities in the development of the macrogametophyte are produced by changes in the internal genic structure or are a response to the influence of extreme environmental stimuli? Statement of some earlier observers (see Focke, 1881) that from the crosses between two white *stramonium*-forms violet *tatula*-forms will be present in the progeny, would indicate, under assumption that the material and observations are correct, the hybrid nature of the plants used for experimental work. A critical cytological and genetical study of *tatula*-form is desirable. One may expect in future to offer some evidence for the probable rôle played by the internal changes of genic structure in the production of the *tatula*-phenotype and to approach the question of its phylogeny and taxonomy.

In the absence of summer material and lacking the experimental evidence and cytogenetic analysis of the plant under discussion, it is at present impossible to make a comparison between the two forms *stramonium* and *tatula*, and no attempt can be made to trace the evolutionary course of the past and to establish the taxonomic positition of *tatula*-form. This will be the object of a subsequent study which is already under way. The following pages are, therefore, necessarily confined to a preliminary report. Since no information on the part of the life history of *Datura tatula* L. hitherto has been done, it seemed to the writer that the present observations in this connection should prove of some interest. While dealing mainly with normal development of the macrospores and macrogametophyte, this preliminary account also refers to some abnormalities observed.

Satina and Blakeslee (1935) have recently made a very interesting study of the dyad plants in *Datura stramonium*,

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*) Grown in Beograd Botanical Garden (own observations),

concerned with cytological effects of the recessive „dy“ gene. Owing to the suppression of the second meiotic division, instead of four tetrads two dyads are formed; the micropylar dyad disintegrates, the nucleus of the chalazal one undergoes three successive nuclear divisions leading to the formation of the eight-nucleate macrogametophyte. The nucleus of the functioning dyad contains 12 pairs of chromosomes; these are divided longitudinally, and in consequence the daughter nuclei and all nuclei of the developing macrogametophyte, inclusive the macrogamete nucleus, contain 24 chromosomes each. The authors state at the end of their article, that the action of the gene »is influenced by environmental factors, as shown by the exceptional cases of tetrad formation“. — While a series of most important studies have been made on *Datura stramonium* by Blakeslee and his co-workers, dealing with cytogenetics of this species and its variants, it would seem that a study of *Datura tatula* has apparently been overlooked. The here presented was undertaken in an attempt to give some cyto-embryological data of this plant. Subsequent reports of cytological and genetical studies on *Datura tatula* will, it is hoped, aid in clearing up some of the problems of classification and its evolution.

Informations concerning macrosporogenesis and the development of the macrogametophyte of members of the Solanaceae are rather limited. As early as 1858 Hofmeister briefly reported on the structure of the mature embryo sacs in some solanaceous plants (*Scopolia atropoides, Salpiglossis picta, Hyoscyamus orientalis*). The first contribution dealing with macrosporogenesis and the development of the macrogametophyte in the solanaceous plants is that of Jönsson (1881). He recorded in *Saracha Jaltomato* the presence of a single-celled archesporium, which gives rise to four macrospores, the innermost of them persisting and developing into a typical eight-nucleate or seven-celled embryo sac. This same condition has been later reported for a number of the Solanaceae: *Nicotiana tabacum* and *Cestrum splendens* (Guignard, 1882), *Atropa belladonna* (Souèges, 1907), *Nicotiana tabacum* Deli-Tabak (Palm, 1922), *Hyoscyamus niger* (Svensson, 1926), *Lycopersicon esculentum* (Cooper, 1931) *Solanum melongena* (Bhaduri, 1932). Krüger (1932), in her study of the development of fruits in *Solanum nigrum* and *Solanum lycopersicum* and their chimeras *S. tübingense* and

S. proteus, made some observations on the development of the macrospores and macrogametophytes. She observed that in *Solanum nigrum* the micropylar macrospore of the tetrad persists as the functioning spore, and the same (contrary to Cooper's information) was assumed for *Solanum lycopersicum.* As to the chimeras, Krüger found that the macrogametophyte may derive either from the micropylar of from the chalazal macrospore; the author came to the conclusion that the micropylar macrospore functions in the majority of cases in *Solanum tübingense,* the chalazal macrospore in *S. proteus.* Contrary to the usual condition, Nannetti (1912) for *Solanum muricata* and Young (1923) for *Solanum tuberosum* reported, that the macrogametophyte originates in a manner similar to that characteristic of the lily (*Lilium*-type), i. e. the archesporial cell in these species of *Solanum* functions directly as a macrogametophyte mother cell. Young's report has recently been verified by Rees-Leonard (1935). This investigator could not confirm the observations made by Young, and found, on the contrary, that the method of development of the macrospores and macrogametophyte in *Solanum tuberosum* shows no deviation from the normal type. Nannetti's report that the development of the macrogametophyte of *Solanum muricatum* follows the „tulip"-type, is not suggestive. There seems no doubt that this observer, lacking the sequence of developmental stages, misinterpreted the structures he observed.

Materials and Methods

The present contribution is based on the material obtained from plants grown in the field in the Botanical Garden at the Beograd University. The material was gathered late in the fall of 1927. Flower buds were collected at different stages of maturity and at some intervals of the day. Whole flower buds, in the case of the youngest ones, were dropped into the fixing fluids; in handling older buds, the ovaries were dissected out and their tips cut away to insure the best possible penetration of fixing agents. Carnoy's acetic-alcohol, Allen's modification of Bouin's fluid, and Zenker's solution were employed as fixatives. The usual cytological technique was followed in imbedding the ovaries in paraffin. Serial microtome sections were stained either in Heidenhain's iron-alum haematoxylin or in a combination ot the latter with eosin or safranin as counterstains.

Development of the Macrosporangium

A cross section through the ovary of ***Datura tatula*** (Fig. 1a) shows that it is made up of two united carpels; but not infrequently three carpels were found to participate (Fig. 1c). The cavity in the ovary is divided into two (or three) locules, one in each carpel. The partition which separates the locules from each other is formed by the inturned margins of carpels. Each locule is in turn, by a secondary septum (s.s) longitudinally divided into two smaller ones, with the exception of the upper part of the locule (Fig, 1b) which remains undivided. As a result of this development and partition, four (or six) placentae are formed; these are, at their distal end, flattened out and broadened.

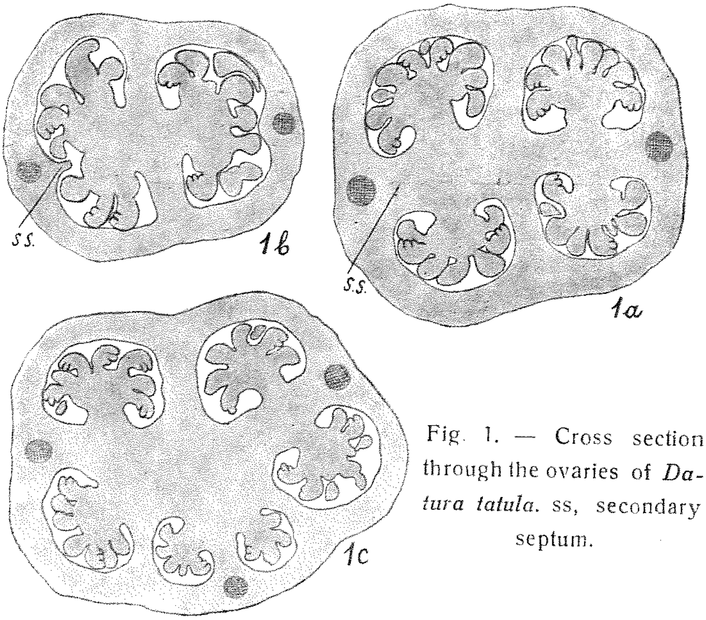

Fig. 1. — Cross section through the ovaries of *Datura tatula*. ss, secondary septum.

Numerous macrosporangium primordia arise from the meristematic placental tissue, and are pushed out as mammillate protuberances into the ovarian cavity. The young macrosporangia grow at first evenly and are of orthotropous form; but as the development proceeds, an uneven growth on the opposite sides causes them to bend toward the placenta. The curvature occurs regularly in a definite direction; some macrosporangia curve toward the secondary septum, the others curve in the opposite direction. As a result of the curvature, the mature macrosporangia become inverted, with the micropyle pointing toward the placenta. But they are not typically anatropous, since they take on a slightly curved form; the macrosporangia suggest a transition toward the campylotropous form, and may be, therefore, designated as amphitropous. This same condition has been reported or figured for a number of the solanaceous plants which have benn investgated up to this time, e. g. *Solanum tuberosum* (Young, 1923; Rees-Leonard, 1935), *Datura metel* (Glišić, 1928), etc.

First indication of the single integument appears at about the time the archesporial cells are well differentiated and are entering the early heterotypic prophase stage (Fig. 2). The integument grows as an annular outgrowth around the nucellus, and by the time the macrosporocyte is in the synizesis stage, it reaches the level of the apex of the nucellus (Fig. 3). When the macrosporocyte is in the heterotypic metaphase stage, the integument has grown over the apical end of the nucellus and it has formed a narrow pasage way, the micropylar canal, of some length (Fig. 4). In mature macrosporangium, enclosing a fully developed macrogametophyte, the integument has developed so far beyond the macrogametophyte as to leave a micropylar canal about 15 cells in length. At that stage the middle part of the integument, surrounding the macrogametophyte, is 6 to 7 cell layers in thickness.

In the central zone of the short funiculus a core of elongated cells is formed which suggests a procambial conducting trace and connects with the vascular system of the placenta.

Macrosporogenesis

At an early stage in the development of the macrosporangium, about the time when first evidence of integument formation is shown by a slight annular rise around the nucellus, *one* or *more cells* in the nucellus become distinguished as the archesporial cells. By their relatively greater size, larger nucleus and nucleolus and by their staining properties, these cells are rendred very conspicuous and are easily recognized from the adjacent cells. As stated above, usually one archesporial cell, which is hypodermal in origin, appears in the nucellus; but numerous instances were observed, in which the archesporium involved 2 to 3 hypo- and subhypodermal cells (Fig, 2, 15—18).

Aside from showing a many-celled archesporium, there is still another feature in development of the nucellar tissue which is of special interest. Namely, in young macrosporangia 2 or 3 binucleate cells are present, lying directly beneath the archesporium. These cells, as shown in Fig. 2, exhibit in many respects the appearance of the true archesporial cells. A careful examination of sections from older macrosporangium failed to reveal the presence of these binucleate cells, the unucleate phase having been in some way restored.

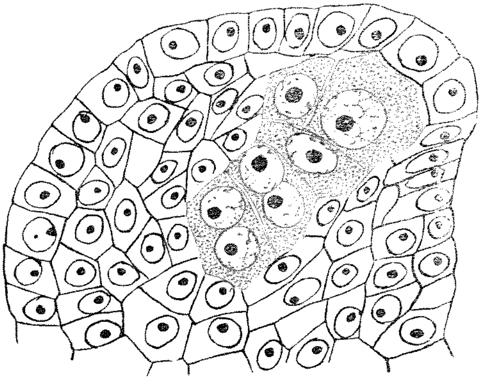

Fig. 2.

Although one not infrequently finds more than one macrosporocyte within the same nucellus to proceed on through the prophase stages of the first meiotic division, there is but one which gains the ascendency and completes its development, resulting in the formation of a tetrad of macrospores. Among the archesporial cells, each of which is potentially a macrospore mother cell, the processes of selection are carried on. A careful examination reveals that the cell to be favoured during this selection is a bit in advance to the others. Fig. 16 shows three archesporial cells in cross section; the favoured one has advanced to the synizesis stage, whereas in other two the development is somewhat retarded. The time at which the selection is completed varies greatly. While some of the supernumerary cells become eliminated at a very early stage of development, the others may continue to compete for a short time, but they have never been seen to progress over the heterotypic metaphase stage. Fig. 18 shows two macrosporocytes side by side; one of them has developed to the macrospore tetrad stage and the three micropylar spores have begun to degenerate, while the additional macrosporocyte, with the chromosomes in the heterotypic equatorial plate stage, has obviously been arrested in development.

Occasionally two further developed macrosporocytes have been observed to lie side by side within the same macrosporangium, as shown in Fig. 26. Among them there is a visible difference in the stage of development, the micropylar in position being evidently the one to be favoured during selection. The favourably situated, in Fig. 26, has developed to the four-nucleate macrogametophyte stage, whereas the neighbouring, with marked signs of degeneration, has been arrested at the dyad stage. One of these dyads contains two nuclei. That the dyads and four-nucleate macrogametophyte in this case are not derived from two macrosporocytes belonging to the same sporogenous tissue, is evidenced by the fact that each of them is surrounded by its own nucellar cells. Such composed macrosporangia have originated by coalescence of the two during their first appearance at the placenta. They may be, therefore, designated as syn-macrosporangia, their nucelli as syn-nucelli. In no case a syn-macrosporangium has been observed, in which more than one macrogametophyte develops to maturity; one of them is tardy and becomes arrested at an early stage of development and ultimately disappears.

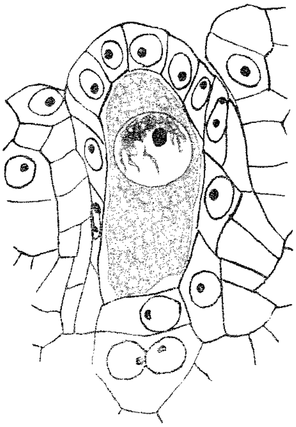
Fig. 3.

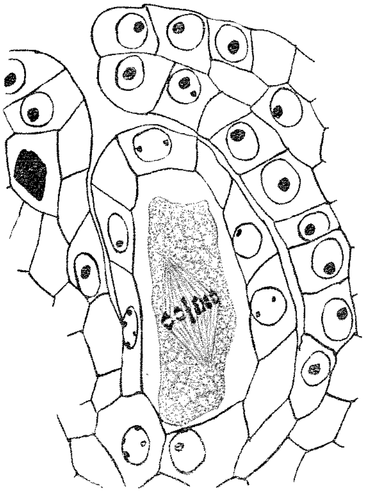
Fig. 4.

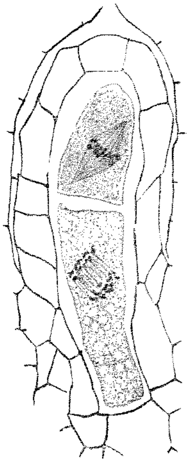
Fig. 5.

Figs. 3—7 illustrate some of the principal stages in macrospores development of *Datura tatula.* The archesporial cell (or cells) does not undergo any preliminary periclinal division to form a primary wall cell and a primary sporgenous cell, but it functions directly as the macrosporocyte. The development of the macrosporangium of *Datura tatula* conforms to the usual sympetalous type (syndermal sensu Dahlgren's, 1928). Prior to the first meiotic division, the macrosporocyte becomes very prominent for its size and form, and is about three times as long as wide. The nucleus is located in the upper portion of the cell; it enlagres greatly in size and, at the onset of the heterotypic division, becomes nearly as large in diameter as the macrosporocyte itself (Fig. 3).

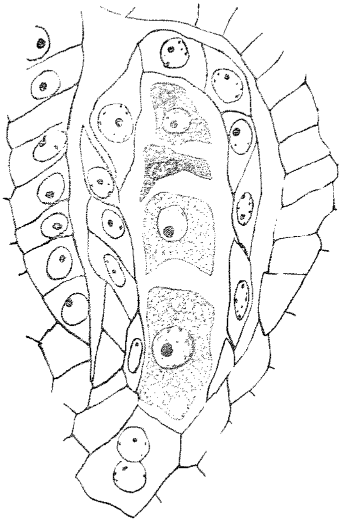

Fig. 6.

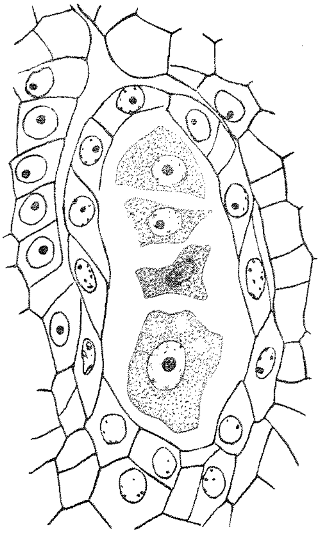

Fig. 7.

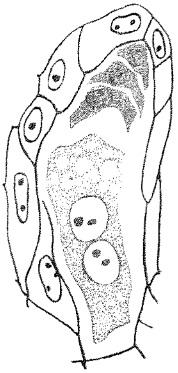

Fig. 8.

The macrosporocyte of *Datura tatula*, as is general rule among the Angiosperms, undergoes two meiotic divisions which occur in rapid succession and result in the formation of a tetrad of macrospores. During diakinesis and at the heterotypic equatorial plate 12 pairs of homologous chromosomes could be plainly seen and counted. There are visible differences in size and form between the various bivalents. In making comparisons of the chromosome complements of *Datura stramonium* (Blakeslee and his co-workers) and *Datura tatula*, one is immediately struck by the identical number and morphological similarity of the cromosomes in the two species.

Following the heterotypic division the macrosporocyte is divided by means of a transverse cell plate into two dyads (Fig. 4, 5), the chalazal cell being somewhat larger of the two. The homoiotypic division, immediately following (Fig. 5), is usually again transverse, so that finally a linear tetrad of macrospores is formed (Fig. 6). However, not infrequently the spindles of the second meiotic division are at an oblique angle to the longitudinal axis of the dyad cells, or the micropylar spindle is transverse, the chalazal one more or less longitudinal. In the latter instances, the meiotic divisions result in the T-shaped tetrad of macrospores, with the upper two lying side by side.

Of the two dyads, the chalazal one is usually the first to proceed on in the second meiotic division, the spindle in the micropylar dyad being lagging a small bit behind the other in appearance (Fig. 5). The fact that dyad stages were relatively scarce in preparations, would indicate that they are of brief duration, the homoiotypic division taking place immediately after the heterotypic division has been completed.

Development of the macrogametophyte

Although any one of the tetrad cells may, for a time, recognize a moderate degree of equallity in the stage of development and have a fair chance of becoming the functioning macrospore, it is the chalazal spore which has a distinct advantage during the competition (Figs. 6, 7). Except in a very few cases to be mentioned later under exceptional cases (Fig. 20), the chalazal macrospore survives and continues to develop to maturity. By three successive nuclaer divisions a normal eight-nucleate or seven-celled macrogametophyte is produced. Therefore *Datura tatula* follows the normal type of macrogametophyte formation.

Prior to the first division of its nucleus into two free daughter nuclei, the functioning macrospore increases considerably in size. During this time the three remaining non-functional macrospores gradually decrease in size; they become more and more distorted in shape until they finally disintegrate. By the time the first nuclear division has been completed and the enlargement of the binucleate macrogametophyte, both in length and in width, taken place, their remnants may be distinguished in preparations as flattened, deeply staining and amorphous bodies above or at the side of the enlarging macrogametophyte

(Figs. 8—10). They disappear entirely about the time when the four-nucleate macrogametophyte stage is reached (Fig. 11); but not infrequently the traces of these spores may be seen as late as the eight-nucleate stage of macrogametophyte (Fig. 12).

After the first nuclear division has taken place, the daughter nuclei move apart, one going to each pole of the cell. In the meantime, the cytoplasm between them becomes vacuolated, and a large central vacuole forms. Furthermore, some smaller vacuoles are present above the micropylar nucleus and a vacuole at the bottom of the cell, directly beneath the chalazal nucleus, may be observed. The present observations could not give any evidence for sustaining the assumphon, that the central vacuole may be active in pushing the daugther nuclei, one to each pole of the cell.

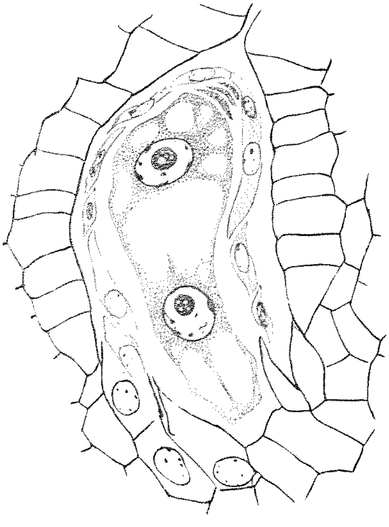

Fig. 9.

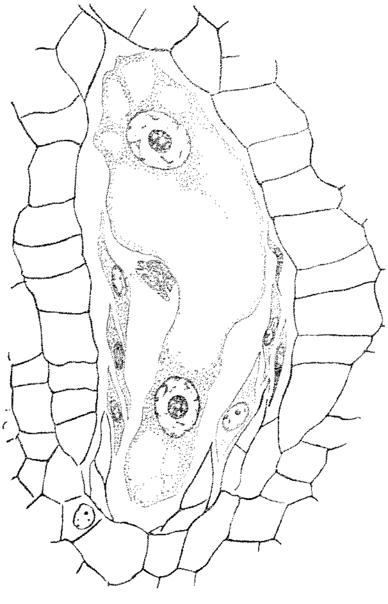

Fig. 10.

Continued growth of the functioning macrospore is followed by degeneration of the surrounding nucellar tissue. The nucellar cells at the sides of the spore begin very early to show signs of degeneration, as early as the meiotic divisions are completed. They become much elongated and pressed against the integument. In a short time thereafter, their contents disintegrate and are finally digested. The nucellar cells at the apex are more resistant and persist for some time, until the binucleate macrogametophyte is formed. Accompanying the disorganization of the nucellar cells, the epidermal cells on the inner side of the integument differentiate into a distinct layer of cells, known as mantle-layer or integument-tapetum (Fig. 6—14). The characteristic shape of these cells is first apparent in the macrospore tetrad stage. They are very conspicuous for their being elongated, perpendicularly to the long axis of the gametophyte; further by their greater size, denser protoplasmic contents and marked meristematic character. As the macrosporangium matures, further changes are taking place in the integumental tissue lying between the mantle-layer and outer epidermis of the integument. These cells become elongated and flattened, their contents gradually absorbed.

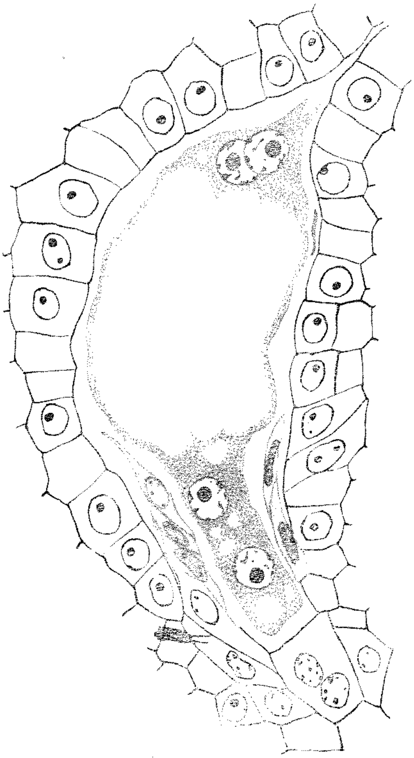

Fig. 11.

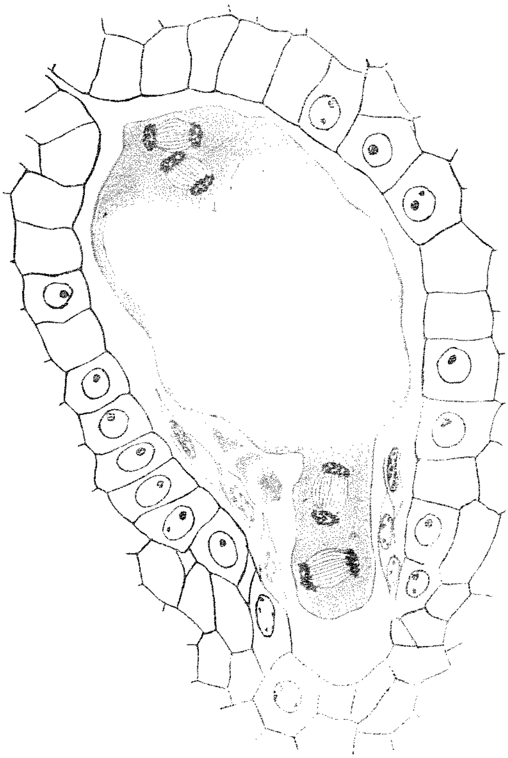

Fig. 12.

As a result of intensive enlargement of the binucleate macrogametophyte, the breaking down of the apical nucellar cells occurs (Fig. 10). After the nucellar apex is ruptured, the young macrogametophyte, its micropylar portion, protrudes from the nucellus and enters the micropylar canal. Its further development is taking place in direct contact with the inner cells of the integument. The fragments of nucellar cells, surrounding the basal portion of the macrogametophyte, remain visible until a much later period of development (Fig. 14). The emerged portion expands considerably in breadth, whereas the chalazal portion, enclosed by remnants of the crushed nucellar cells, remains constricted and tubular. The macrogametophyte becomes flask-shaped, assuming the labiateous or scrophulariaceous appearance. In accord with the amphitropous form of the macrosporangium, the mature macrogametophyte of *Datura tatula* becomes slightly curved.

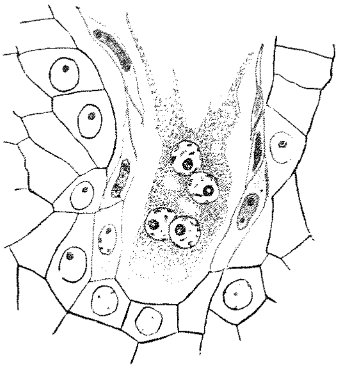

Fig. 13.

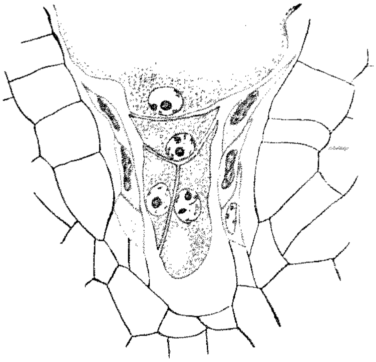

Fig. 14.

The mycropylar and chalazal nuclei of the binucleate macrogametophyte undergo now a new division and so the four-nucleate phase is reached (Fig. 11). The nuclei divide simultaneously. The two spindles lie approximately at right angles to each other; the micropylar spindle, more or less horizontal, is located in the apical end of the cell, the chalazal one is parallel to the long axis of the cell and forms in the constricted portion, at the level of the disorganized nucellar apex. In consequence of this orientation of spindles, the two mycropylar nuclei are arranged side by side, the two at the chalazal end are one above the other.

The last (third) nuclear division, giving rise to an eight-nucleate macrogametophyte, was frequently observed. The division of nuclei occurs simultaneously, as shown in Fig. 12. The two spindles in the micropylar end are at right angles to each other, those at the chalazal end also. The two tetrad groups of nuclei are separated by a very large central vacuole. They soon become organized into a definite seven-celled macrogametophyte, the organization of the antipodal apparatus (Fig. 14) being a small bit in advance to the egg-apparatus. The polar nuclei move toward each other; they fuse together prior to fertilization to form a large nucleus of the endosperm initial cell. The fact that the polar nuclei move toward each other after a very large central vacuole between the two tetrad groups of nuclei is formed, suggests that the nuclei are capable of autonom movement (attraction!).

Exceptional cases

1) ***Multiple macrosporogenous tissue.*** -- The occurence of more than one archesporial cell within the same nucellar tissue (Figs. 15—18) is of a such frequency, that it may not be strongly regarded as an exceptional condition. As previously stated, all of the cells of the multiple archesporium are potentially capable of further development, and show more or less a tendency to persist and to function. Their development, however, does not proceed uniformly. There is always but one of them which has a distinct advantage during competition; this cell becomes the survivor, the additional ones being tardy and soon

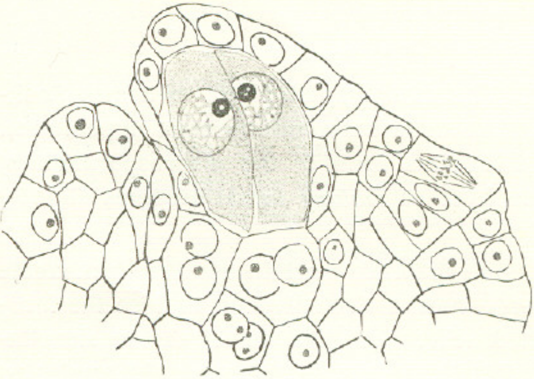
Fig. 15.

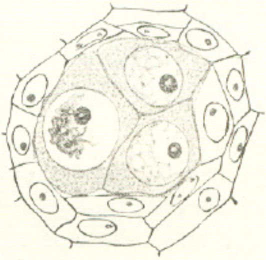
Fig. 16.

arrested in their growth. The eliminated cells become aborted, they disintegrate and are ultimately digested in the processes of development of the functioning archesporial cell. In no case the supernumerary macrosporocytes have ever been seen to go over the heterotypic equatorial plate stage (Fig. 18).

A many-celled archesporium has been recorded for a number of the solanaceous plants. Lesley (1926) found occasionally two macrosporocytes in *Solanum lycopersicum*, Bhaduri (1932) reported a multiple archesporium in *Solanum melongena*, Rees-Leonard (1935) observed six archesporial cells in one macrosporangium of *Solanum tuberosum*. Since a many-celled sporogenous tissue is generally found to be confined to certain more primitive families, the occurrence of this primitive character in a such highly specialized family as the Solanaceae would seem especially noteworthy. These observations lead toward the idea that the Solanaceae are not too far removed from the families exibiting normally such primitive condition. In the writer's former paper (Glišić, 1928) on the endosperm formation of *Datura metel* L., it was pointed out that the family of the Solanaceae is to be considered as a primitive family among the Tubiflorae. This assumption based chiefly on the presence of

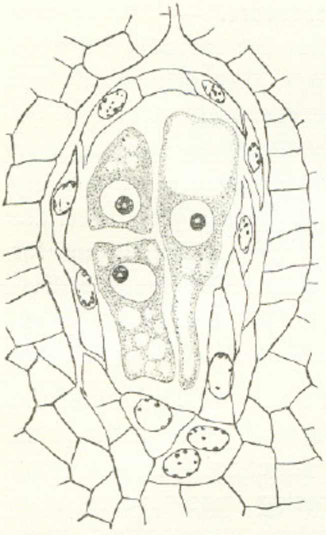
Fig. 17.


Fig. 18.

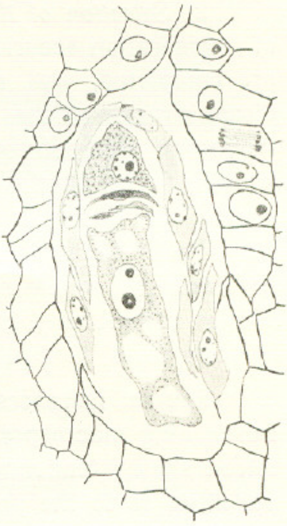
Fig. 19.

different types of endosperm formation exhibited by different members of the family (*Datura*—cellular; *Hyoscyamus*—helobial; *Schizanthus*—nuclear). The present observations on the archesporium of *Datura tatula* and those of other investigators on other members of the Solanaceae, may be of some value in submitting additional data to the above assumption. It was repeatedly demonstrated that the results obtained from cytological, embryological and genetical invesitgations are helpful in interpreting some of the problems of classification and of phylogenetic evolution. Therefore, a more complete study of a larger number of the solanaceons plants is needed.

2) *Selection of the functioning macrospore.* — The process of degeneration occurring immediately after the meiotic divisions are completed, and resulting in complete disorganization and disappearance of the non-functional macrospores, does not involve the members of a tetrad at the same time. Degeneration usually begins at the micropylar end and expands progressively toward the chalaza. The products of the micropylar dyad are the first to show marked signs of degeneration. Indications for that may be noted as early as before the second meiotic division is completed (Fig. 5). This division in the micropylar dyad, in such cases, is lagging a bit behind that in the chalazal dyad.

Several instances have been observed, in which besides the functioning macrospore one or two additional spores of the same tetrad show a tendency to persist and to function. There is some variation as to the position of the competing macrospores. In a number of cases the innermost and apical macrospores were found to compete, the other two in the middle presenting already a completely disorganized appearance (Fig. 19). While the elimination of the three upper macrospores is the rule, in extreme cases the three lower ones were seen to disintegrate, the apical spore remaining to function. This exceptional condition, as shown in Fig. 20, has been encountered in preparations only once.

From these observations may be understood, that any one of the macrospores is potentially capable of giving rise to an eight-nucleate macrogametophyte. All four macrospores have a fair chance of becoming functional. But it is the innermost of them which is more favourably situated and destined to survive and to proceed normally in further stages of the macrogametophyte development to maturity. The process of selection is completed at a relatively early stage of development, before the first nuclear division of the selected spore has taken place. The functioning spore is distinguishable from the very beginning; it is always a little in advance to the others.

3) *„Triads“, „dyads“ and „monads“.* — Several instances were observed, in which the formation of a tetrad of macrospores has been omitted. Instead of four, a reduced number of cells are produced.

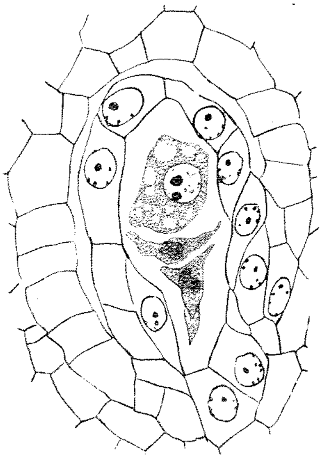
Fig. 20.

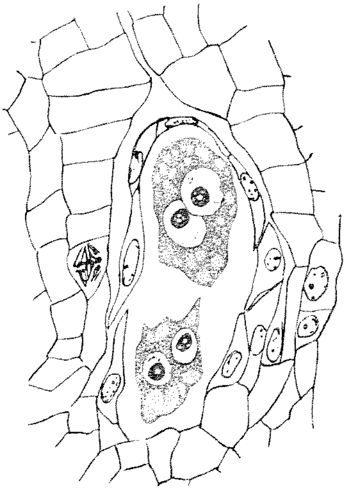
Fig. 21.

a) Very often the micropylar dyad was seen to degenerate at an early stage, before the initiation of the second meiotic division, whereas the chalazal dyad normally completed its division into two macrospores. An early degeneration of the micropylar dyad is found in *Solanum tuberosum,* as reported by Rees-Leonard (1935). Occasionally an aborted spindle of the second meiotic division is to be seen in the micropylar dyad. Fig. 5. shows a such case of early degeneration of the micropylar dyad. As a result of these processes three cells or „triads“ are formed, among them one dyad (toward the micropyle) and two chalazal macrospores.

b) Not infrequently the second meiotic division was completed, but it was not followed by cytokinesis. As a result two binucleate cells or „dyads“ are formed (Fig. 21). From the normal macrospores the binucleate „dyads“ differ in that they have the value of bispores, each nucleus corresponding to that of a macrospore nucleus. In some instances the micropylar dyad was found to degenerate before the second meiotic division of its nucleus occurs; in another case the micropylar dyad remained uninucleate, whereas the chalazal one contained two nuclei (Fig. 22) or, in some cases, an aggregate of nuclei of different size (Fig. 23).

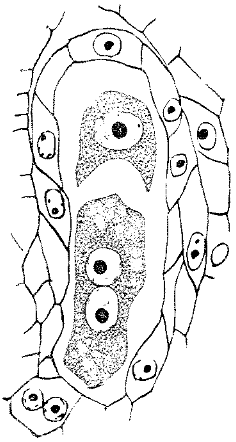
Fig. 22.

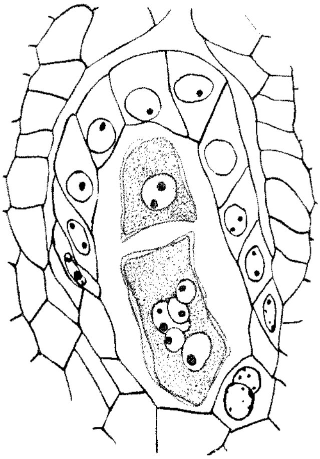
Fig. 23.

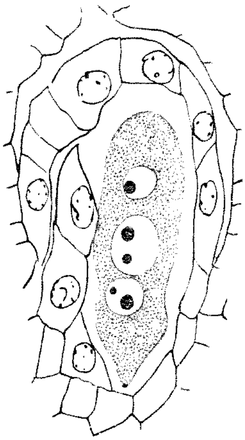
Fig. 24.

c) In one extremely exceptional case the divisions of the macrosporocyte nucleus were not followed by cytokinesis, and in consequence instead of four cells only one multinucleate cell was found. No trace that would indicate the presence of tetrads or dyads could be detected. Fig. 24 shows a such cell containing three nuclei. These nuclei may be homologized with the dyad and tetrad nuclei. A such cell may be designated as „monad“. In this connection may be quoted, that a similar case has recently been reported by Anderson (1933) for Red Elongate, a primary trisomic variant of *Oenothera franciscana.*

— The structures here recorded, resulting from a failure of karyo- or cytokinesis, present a abnormal appearance and there is no doubt that they are not viable. Sooner or later a cessation of development occurs and degeneration begins. In no case such abnormal structures have been seen to proceed so far in development as to the mature gametophyte. They are arrested in their development, as indicated by their unelongated condition. Many cases have been seen, in which the four-nucleate megagametephyte was very small, with remains of the three non-functional spores at the micropylar end, and all surronnded by an intact nucellus (Fig. 25). The causes of this inhibited and abnormal condition are not known. In the same ovarian cavity may be seen the macrosporangia at different stages of development; besides the macrosporangia with an eight-nucleate macrogametopyte are to be observed those with dyads or tetrads. The multipartition of the dyad nucleus, as stated above, is doubtless an evidence of a pathological condition of the cell. The abnormalities which occur during the development of the female gametophyte of *Datura tatula* may be due to the influence of environmental stimuli. That the environmental factors may play an important rôle in producing developmental abnormalities it has been admitted and experimentally shown by many investigators. But it is not excluded that hybridity may as well acount to a certain degree for abnormalities observed.

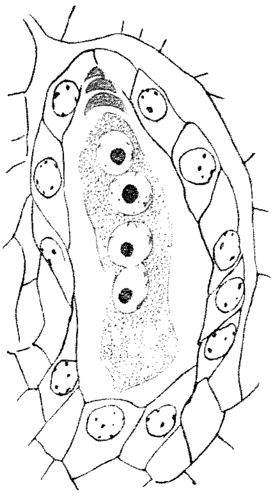

Fig. 25.

4) *Binuclearity.* — The occurrence of the binucleate cells immediately below the archesporium in *Datura tatula* has generally been observed (Fig. 2). These cells were seen only in young nucelli. As far as the writer is aware, the binuclearity in the nucellar tissue has not been recorded up to this time. Whether the binucleate condition here may be considered as a normal phase in tissue development, as supposed by some investigators (Beer and Arber, 1915, 1920; Prankerd 1915) or this condition may be explained in another way (see Wareham, 1936), it is an open question. The origin and fate of these binucleate cells is not clear. In older tissue there is no binucleate cells, the uninucleate condition having been gradually restored in some way.

5) *Syn-nucelli.* — In several instances two macrogametophytes were observed to develop within the same macrosporangium. In one case the chalazal macrosporocyte was in dyad stage (in another in tetrad stage), whereas the adjacent structure was a macrogametophyte at the binucleate stage (Fig. 26). An examination of these cases showed, that both structures were separated from each other by a distinct layer of flattened nucellar cells, an evidence that these structures may be regarded as two nucelli, which have been united (grown together) at a very early stage of development, before the integument appeared. Therefore they may be designated as syn-nucelli.

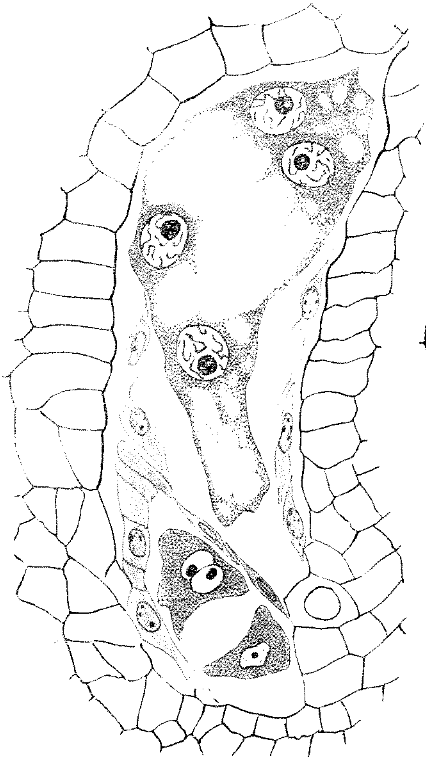

Fig. 26.

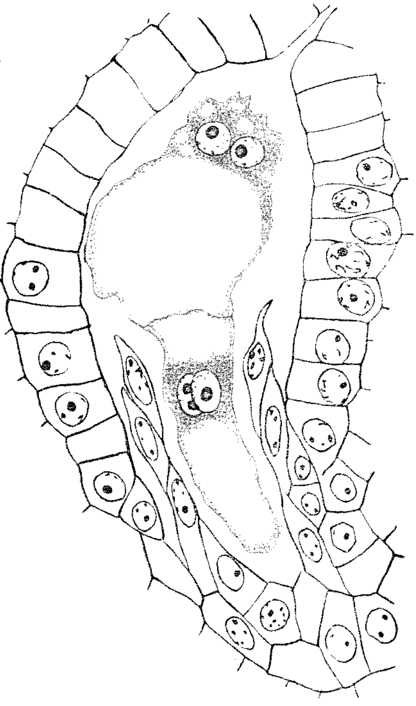

Fig. 27.

6) „*Endosperm-like division*". — In some very few cases a distinct transverse membrane was seen to divide the four-nucleate gametophyte into two binucleate portions. As shown in Fig. 27, the two separate protoplasmic portions appear to be in a healthy, normal condition. Origin and significance of this exceptional condition could not be understood.

Summary

1) The ovary consists of two (or three) united carpels. By the inturned margins of carpels two (or three) primary locules are formed, one in each carpel. Each locule is further by a secondary septum divided into two smaller ones.

2) Numerous anatropous macrosporangia with a slight transition to campylotropy are borne on the enlarged placentae. The macrosporangia are of amphitropous form.

3) Besides a single-celled archesporium, frequently a multiple archesporial tissue is present.

4) By the two successive meiotic divisions a tetrad of macrospores, arranged in a linear row, is formed. Occasionally a T-shaped form of tetrad has been encountered. The additional macrosporocytes have never been seen to go over the heterotypic metaphase stage.

5) The innermost macrospore persists and continues to develop into the mature macrogametophyte; the other three spores disintegrate. Only in one case the micropylar macrospore was found to persist.

6) A typical eight-nucleate or seven-celled macrogametophyte is formed. The nucellar cells at the sides and apex break down and the macrogametophyte completes its development in direct contact with the mantle-layer (integument-tapetum).

7) A number of exceptional abnormal cases were observed The occurrence of „triads“, „dyads“ and „monads“ among these abnormalities are especially noteworthy. The question is open as to the causes of the abnormal condition. They represent structures produced by inhibition.

8) The occurrence of binucleate cells directly beneath the archesporium has been noted. In older stages the uninucleate phase is restored in some way. Origin and significance of these binucleate cells remained unknown.

9) Syn-nucelli, originating by union of the two during the first appearance of the macrosporangium primordia at the placenta, have been frequently encountered. In every case only one of them develops to maturity.

10) In very few instances a transverse membrane was observed to separate two binucleate portions of a four-nucleate

macrogametophyte. Origin and significance of this „endosperm-like“ membrane is not known.

11) The occurence of a number of primitive characters, such as many-celled archesporium and different types of endosperm formation, would indicate that the family of the Solanaceae is to be considered as a primitive one among the Tubiflorae.

(From the Botanical Institute and Garden of the Faculty of Philosophy, University of Beograd).

December, 1936.

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