Modilewski, I. 1928. Die Embryologische Entwicklung von *Thesium intermedium*. *Bull. Jard. Bot. de Kieff* 7: 65-70.

The Embryological Development of Thesium intermedium.

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The embryological observations on *Thesium* species are partly very much outdated and partly incomplete. If Guignard in his work on *Thesium divaricatum* (1884) described the facts in detail, then one must attribute the characteristics of *Thesium intermedium* to incomplete short remarks by Jönsson. The purpose of this short article is to communicate the results of my re-examination of *Thesium intermedium* and to add some additions to the older work.

I collected the material over the years 1925-1926. As a fixative medium I used formalin-chromic acid and alcohol-glacial acetic acid. For staining iron haematoxylin and pianese were mostly used.

In the recent stages of development of the ovaries, it is known that three ovules attached to a common funiculus develop in *Thesium intermedium*. The latter grows quite fast and takes on the length the ovarian locule, therefore the funiculus experiences a turn because of lack of area around its axis and accepts a screw-shape, the ovule is at the same time pressed in slightly on the ovarian pericarp in the upper parts of the ovarian locule.

In the earlier stages all three ovules develop identically and at the same time; only in somewhat older stages of development do the two ovules begin to degenerate and as a final result only one ovule develops further. As a characteristic the emergence of a clearly marked archesporiumium is seen in the young ovule of *Thesium intermedium*. The archesporium usually develops at the beginning from only in the number of three cells, to which gradually new and new archesporium cells are added and finally their number rises up to nine to eleven. All these cells are characterized by their relationship-large in comparison with the remaining cells, which are generally very small with *Thesium intermedium*. The nuclei of the archesporial cells point to different stages of the reduction division because of the unequal age of the latter (Fig. 1-2).

It is necessary to point out that the arrangement [composition, construction] takes place directly from the first archesporial cells in the young ovule under the epidermis layer. Thus the archesporial cells separate no tapetal cells and transform directly into the embryo sac mother cells, those embryo sac mother cells, which develop later on the sides of older ovules, or are put on more deeply down in the ovule.

Not rarely does one observe large cells beside the mentioned embryo sac mother cells, which are, however, of vegetative origin since their nuclei do not exhibit a typical prophase reductional division.

My observations of *Thesium intermedium* differ from the observations which were made by Jönsson at that time. Due to my investigation I am inclined to interpret some of Jönssons' assertions as incorrect. In complicated conditions which develop because of the successive development of the archesporium in *Thesium intermedium*, it serves as only a safe criterion for differentiating archesporial cells and somatic cells in the ovule, the stages of the nucleus, i.e. whether it is subject to atypical or typical nuclear division.

Among the other *Thesium* species, *Thesium divaricatum* was examined embryologically by Guignard. The author writes: "the large subepidermal cells are primordial mothers cells of the embryo sacs, the longitudinal section shows as often two of them as one, very seldom of advantage" ["Les grandes cellules sous-épidermiques sont des cellules mères primordiales de sacs embryonnaires, la coupe longitudinale en montre aussi souvent deux qu'une, très rarement d'avantage".]

From this clue [point, evidence] it follows that with *Thesium divaricatum* no tapetal cells are put on, and that the number of the embryo sac mother cells refers to the arrangement of an archesporium, although the latter is not so strongly pronounced as with *Thesium intermedium*.

All nuclei of the young embryo sac mother cells exhibit prophase reductional divisions, while in some cells prophase remains located in the nuclei at the dynapsis stage, the nuclei of other cells arrive in their development up to the spirem stage and advance still further to other stages. The creation of the reductional divisions of the nuclear spindel one usually observes in two embryo sac mother cells. As a consequence of this development, the embryo sac mother cells occurring at different times develop directly one or two young embryo sacs, which do not form daughter cells. In this case, if two embryo sacs develop, only one develops further, the other one usually dies at the four nucleate stage.

Concerning the formation of the daughter cells from the embryo sac mother cells, then *Thesium divaricatum* and *Thesium intermedium* differ substantially from each other in this relationship. From the statement by Guignard, three daughter cells develop in *Thesium divaricatum*. The author writes: "Almost always, when there are two primordial mother cells only one continues its development, and is subdivided into three superimposed daughter cells, whose lower one appears larger at the beginning and increases quickly to give the embryo sac". ["Presque toujours, quand il y a deux cellules meres primordiales une seule continue son developpement, et se subdivise en trois cellules-filles superposées, dont l'inférieure apparait plus grande des le debut et s'agrandit rapidement pour donner le sac embryonnaire".]

Sometimes one observes with *Thesium intermedium* beside the embryo sac mother cells vertical rows of three to four cells positioned one above the other, whose size and nucleus, however, clearly point to to their somatic origin. Apparently such small columns of cells develop at the same time beside the young embryo sac, which grows rapidly in length. (Fig. 2).

At same time begins, with the further development of the embryo sac in one of the three ovules, all remaining embryo sac mother cells in the archesporiumium degenerate and the end result is only one ripe embryo sac occurring in one of the ovules. In the two remaining ovules at the beginning of their development the processes are carried out in the way described above, but later not only the neighboring embryo sac mother cells degenerate, but also the young embryo sac, which usually does not develop further than up to the 4 nucleate stage. Thus finally only one ripe embryo sac is present in the ovary, although at the beginning three- or more-celled archesporia were put on. (Fig. 3).

The development of the embryo sacs proceeds in the typical way. The only deviation consists of the fact that the three lower nuclei disappear extraordinarily fast. It is nearly impossibly to observe the eight nucleate stage. Probably remaining only for a moment, if the lower four nuclei develop, only the polar nucleus moves from the center of the embryo sac, while the three remaining disappear completely. During the study of the development of the embryo sacs of *Thesium intermedium* several times I received the impression, as if in lower parts of the embryo sacs in individual cases no nuclear tetrad develops at all, but only two nuclei are possibly present here, of which one functions as a polar nucleus and the other one rapidly goes to the base (Fig. 4-5).

Similar conditions are not inherent only to *Thesium intermedium*, but also *Thesium divaricatum*, about which Guignard writes: "one finds sometimes states similar to that of figure 13 (in which two nuclei in the upper and a nucleus in the lower end of the embryo sacs are represented), where, the nucleus at the base remains undivided, while that at the top was divided. Subsequent divisions are very difficult to follow because of the smallness of the nuclei, the antipodals are not constituted with the state of the cells at the bottom of the sac, their nuclei disappear very quickly”. [“on trouve parfois des etats semblables à celui de la figure 13 (an welcher zwei Kerne im oberen und ein Kern im unteren Ende des Embryosackes dargestellt sind), où ‚le noyau de la base est resté indivis, tandis que celui du sommet s'est partagé. Les divisions ulterieures sont tres difficiles à suivre en raison de la petitesse des noyaux, les antipodes ne se constistuent pas à l'état des cellules au fond du sac, leurs noyaux disparaissent très rapidement”.]

Both polar nuclei merge usually before fertilization, and form the secondary embryo sac nucleus. The egg apparatus consists of the ovule, whose characteristic mature traits are missing, and of two small synergids. Thus the ripe embryo sac contains the ovule, two synergids and the secondary embryo sac nucleus. The ovule and even the whole egg apparatus attach themselves, depending upon maturation of the embryo sacs, asymmetrically onto upper parts to the latter; thereby supporting the ovule on the side, which either to the placenta or to that, which is arranged to the ovarian locule. At the same time the antipodal part of the embryo sac begins to bend in the direction of the placenta and this excrescence contains little protoplasm (Fig. 6).

At the same time the degeneration of the archesporiums in the two remaining ovules ends. These destroyed cell complexes stand in connection with those ovules, which the mature embryo sac contains (Fig. 9). Going parallel to the mentioned processes as well as those cells, that the vascular bundle of the funiculus accompanied destroyed. But runs the full connection of all cell complexes of the remaining two ovules and the placenta degenerated with usually mature embryo sac to a system in those cases, where fertilization took place. As a consequence of fertilization the haustorium extends rapidly and grows deeply down into the funiculus in the shape of a long narrow channel.

At the same time the development of the endosperm begins. Already present are the first two endosperm nuclei in the embryo sac, which are separated immediately from each other by a cell wall, while in the nucleus of the ovule the combination of the sexual elements cannot yet be completed: Generally the ovule experiences after fertilization a longer quiescent period, during which several endosperm nuclei in the embryo sac develop, and which divides the whole embryo sac into some cells. Up to this time the top of the embryo sac comes directly in contact with the ovarian locule. (Fig, 7-8).

It is characteristic for *Thesium intermedium* that only one considerably large haustorial nucleus emerge in the lower part of the embryo sac. It develops in the same way as was described by Guignard for *Thesium divaricatum*. The secondary embryo sac nucleus divides into two primary endosperm nuclei, which are approximately polar positioned; thereby the embryo sac is divided into an upper and lower cell. While the upper cell and its nucleus incur further divisions, the lower nucleus experiences no more divisions. It becomes larger rather rapidly; at the same time the lower endosperm cell also becomes larger, which contains its nucleus and transforms gradually into the long haustorium (Fig. 7-9).

In connection with the physiological role, which comes to this haustorial nucleus, it incurs large changes in its structure and at the time as its particularly intensive functioning attains the following shape. With significant enlargement of this nucleus the demarcation of its plasma membrane surrounding it is clearly lost. The outside outline of the nucleus receives a very irregular queer form, it develops on its surface recesses, folds and raised areas, which lead to the enlargement of its surface; its nucleolus, usually singular in existence, achieves a significant circumference, takes on an irregular shape and contains some vacuoles. The chromatin concentration of the nucleus is clearly visible in the form of some small lumps. This haustorial nucleus, which develops during the first division of the secondary embryo sac nucleus, remains during the whole development of the embryo and is even still visible when the embryo has reached nearly full ripeness. In the cytoplasm, which surrounds the haustorial nucleus, clearly the mitochondria are visible. The cytoplasm exhibits cytoplasmic streaming. (Fig 10-11).

Because of the extraordinarily small dimensions of the nuclei and the chromosomes the cytological investigation of *Thesium intermedium* is a little fruitful task. Suitable stages of the pollen development were missing to me; the pictures of the reduction division in the embryo sac mother cells also did not provide much on the designation of the chromosome number and only in some endosperm cells were the division figures of the nuclei suitably clear for counting. I counted 34-35 chromosomes, apparently they are 36, from this follows that the haploid number-12, which is diploid 24. One can indicate still another peculiarness, which consists of the fact that during the division of the endosperm nuclei the nucleolus remains still at the metaphase stage. (Fig. 12).

Literature.

Jönsson B. Om embryosäckens utveckling has Angiospermerna Lunds Univ. Arskrt Bd. 16. 1881.
Guignard L. Observations sur les Santalacées. Annales de Sciences naturelles. Tome 2. 1885.

Figure explanation.

Fig. 1. Two sections from the same series of a young ovules with three archesporial cells.
Fig. 2. Four sections from the same series of an older ovule with 11 archesporial cells.
Fig. 3. Two sections from the same series of a ovule with a four-nucleate embryo sacs, with a three-nucleate and with some degenerating embryo sac mother cells.
Fig. 4. Eight-nucleate embryo sac.
Fig. 5. Embryo sac with the egg apparatus and with both polar nuclei.
Fig. 6. Ripe embryo sac with the egg apparatus and with secondary embryo sac nucleus.
Fig. 7. Embryo sac with the fertilized ovule and with the two first endosperm cells.
Fig. 8. Embryo sac with the fertilized ovule, with several endosperm cells and with the haustorial cell.
Fig. 9. The emergence [formation] of the haustorium.
Fig. 10. The haustorial nucleus.
Fig. 11 The haustorial cell with the mitochondria in the cytoplasm.
Fig. 12. Division of the endosperm nuclei.