

**MACROSPOROGENESIS, DEVELOPMENT OF FEMALE
GAMETOPHYTE AND BEGINNING EMBRYO DIFFERENTIATION
IN SOME MEMBERS OF THE FAMILIES VITACEAE
AND LEEACEAE, FOLLOWED BY SOME PHYLOGENETIC
REMARKS ***

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The present study includes the original description of some particulars of the macrosporogenesis, of the female gametophyte development, of the fecundation process, of the initial differentiation of the nuclear-type secondary endosperm, and of the embryo differentiation in 11 taxonomic units belonging to the family Vitaceae and in one Leeaceae species.

The fecundation time was determined in *Vitis silvestris* and in two grape varieties. In contrast with literature data, the existence of the antipodes was also demonstrated during and subsequent to the fecundation phenomenon, as well as an increase of their volume and their multiplication.

The present paper includes some phylogenetic considerations on the Rhamnales Order, based upon original caryological, morpho-palynological and embryological data correlated with the information in the literature.

The family Vitaceae caught the attention of many investigators since long ago, due to the practical-economical and the ornamental value of its members.

* This work represents the embryological part and general conclusion of the thesis for a doctor's degree sustained in 1968 under the title „*Caryological, morpho-palynological and embryological investigations in the Vitaceae s.l. (including the Leeaceae)*“. To the above, morpho-palynological observations on several members of the family Rhamnaceae are presently added, as well as general considerations on the Rhamnales order, based both upon personal and literature data. The remaining chapters of the above mentioned thesis, such as the caryology, morpho-palynology and male gametophyte development, were published in 9 notes (see Literature List, 64—69, 89—91).

The systematics of the family was controversial for quite a long time. Until 1789, when in Jussieu's conception genus *Vitis* together with genus *Cissus* made up the Order Vites, the various genera nowadays included in this family were adjoined to others belonging to different families, more or less phylogenetically related. Jussieu perceived among the first genus *Vitis* relationship to the family Rhamnaceae on account of its floral morphology. Bartling (1830), grouped under the name of Sarmantaceae both Jussieu's Vites and the Leeaceae, and Spach (1834) shared this view.

The name of Ampelidaceae originates in Kunth's papers (1821) and was taken over by De Candolle (1824), who placed in his new order Ampelideae two tribes: Viniferae or Sarmantaceae and the Leeaceae.

The denomination of Vitaceae is due to Lindley (1836), who included in it genera which constitute today the families: Olacaceae, Francoaceae, Sarraceniaceae and Pittosporaceae.

A. Engler (1892, 1897) conceived the Rhamnales Order in its strictest sense, limiting it to the families Rhamnaceae and Vitaceae (genus *Leea* included), taking into account the morphology of the flower and, in particular, that of the ovule. Before Engler, the Rhamnales were considered to comprise numerous families more or less related.

There existed other opinions still on the systematic position of the family Vitaceae, thus, for instance, Van Tieghem (1898) placed both the Vitaceae and the Rhamnaceae within the order Celastrales, along with the Celastraceae, Illicaceae, Impatiaceae, Platanaceae, Violaceae

Baillon (1877) inserted the Rhamnaceae after the Celastraceae, while the Vitaceae (*Vitis* and *Leea*) into the Loranthaceae 'series', the latter being built up from most heterogeneous groups.

Kerner (1891) completely separated the two families, placing the Ampelidaceae in the 'Discophorae Branch' (which comprised most of the Geraniales and Sapindales), while the Rhamnaceae in the 'Crateranthae Branch'.

The Rhamnales were again conceived in a wider sense by L. Beille (1902), who attached to them, besides the Rhamnaceae and the Ampelidaceae, the families of Celastraceae, Staphyleaceae and Illicaceae, in consideration of the position of their stamens, as well as of the pathways of their vascular fascicles.

In the system of August Heintze (1927) the Vitaceae and the Rhamnaceae are included in the Meliales, along with the Meliaceae, Rutaceae, Anacardiaceae, etc.

Hutchinson (1926) presented the order Rhamnales in an altogether different conception, adjoining namely the Rhamnaceae and the Ampelidaceae (Vitaceae) to the Elaeagnaceae and Heterophyridaceae, that is to families ascribed by Engler to the Myrtiflorae.

Engler's conception (1892, 1897) was adopted much later. A. B. Rendle (1925) and Wettstein (1935) attach this small order containing only two families, the Rhamnaceae and Vitaceae, to the Gruinales, Terebinthales and Celastrales.

In the modern phylogenetic classification systems the Rhamnales Order is considered as deriving from the Celastrales Order, or evolving

concurrently and in parallel to it, as an ultimate point of evolution: Wettstein (1935), Busch (1940), Pulle (1592), Taktajan (1959), Emberger (1960), Soó (1961).

Hutchinson (1959) derives on the one hand from the Thiales the order Celastrales, thence the Euphorbiales and then the Rhamnales, and, on the other, from the same Thiales the Malvales, then the Euphorbiales, wherefrom the Rhamnales evolved. Further, Hutchinson does not consider the Rhamnales as an evolution ultimate point, but derives from in the Myrsinales and hence the Ebenales.

Order Rhamnales, with its three families: Rhamnaceae, Vitaceae and Leeaceae, is considered by many botanists as an independent order, as a natural enough order — thus Engler (1892), Gagnepain (1911), Wettstein (1935), Suessenguth (1953), Emberger (1960), Schultze-Motel (1964), — differing from order Celastrales mainly in the disappearance of the outer staminal cycle (episepalous), hence the stamens are located against the petals (epipetalous). However, some botanists — R. Echevin (1964), P. Crété (1965) — place the Rhamnales as a sub-order within the Celastrales, resuming an older conception.

Inside the Rhamnales Order the Rhamnaceae family is well outlined, while the systematics of the Vitaceae family is more difficult and still controversial. The position of genus *Leea*, often included in the Vitaceae family in the rank of a genus or of a sub-family, was much discussed as to whether or not it may constitute an independent family. Many botanists: Engler, Gagnepain (1911), Wettstein (1935), Chadeaud and Emberger (1960), Schultze-Motel (1964), and, in particular, K. Suessenguth in his monograph on the Rhamnales Order, upon a comparative morphological and embryological analysis carried out in the families Vitaceae and Leeaceae (1953), showed genus *Leea* may be raised to the rank of a family and cannot be left in the Vitaceae family, since this would render the latter too unhomogeneous.

Within the family Vitaceae it would be interesting to know whether the much discussed genera *Vitis*, *Cissus* and *Ampelopsis* constitute natural units and whether a multilateral analysis may justify the actual delimitation of the genera *Parthenocissus*, *Ampelocissus*, and *Cayratia*.

The disputed systematics of Order Rhamnales and, in particular, of Family Vitaceae determined us to undertake caryological, morpho-paly-nological and embryological investigations in some plants belonging to this group.

The peculiarities found in the morphological structure of the pollen grains, the characteristic embryological features of the male and of the female gametophyte development, the details of the embryo and of the secondary endosperm formation enabled some taxonomic considerations both within the Vitaceae family and the Rhamnales order.

Material and Methods

In our thesis for a doctor's degree we analysed from a caryological standpoint 27 taxonomic units belonging to 6 genera and representing 18 species, 2 productive hybrids and 7 cultural varieties. The material

was sampled in the Botanical Garden in Bucharest, in the Ampelographic Collection of the Agronomical Institute in Bucharest, and in the Botanical Garden in Bogor, Indonesia.

The conventional method was used (fixing solution: Navashin-Bruun; embedding in paraffin and cutting at 5—7 μ ; staining with hematoxyline according to Heidenhain and Ehrlich), as well as the quick carmine-acetic method according to J. Belling. Mitotic metaphases in the root vegetative tips were analysed.

The morpho-palynology chapter includes analyses of the pollen grain structure in 8 Rhamnaceae species, 31 Leeaceae species and 112 taxonomic units belonging to the Vitaceae family. The material was sampled in the Botanical Gardens in Bucharest, Rumania, and in Bogor, Indonesia, as well as in their herbaria. It was analysed in water and chloralhydrate, and drawings were made thereafter in the light chamber, as well as microphotographs.

The embryological investigations aimed at ascertaining the details of microsporogenesis, the peculiarities in the structure of mature pollen grains, and the characteristic features of male gametophyte development in 20 taxonomic units.

Pollen cultures were made in fluid or solid media, to which glucose, saccharose and fructose were added in a concentration range of 5 to 35%.

The investigations were further pursued in an endeavour to find out characteristic data about the macrosporogenesis, the female gametophyte development, the fecundation process, the beginning differentiation of the nuclear-type secondary endosperm and the embryo differentiation in 11 taxonomic units in Family Vitaceae and in one unit in Family Leeaceae.

The embryological material was fixed in the Navashin-Bruun, Carnoy and modified (by Gerassimova) Carnoy solutions; 5—15 μ thin sections were cut; the preparations were stained with Heidenhain's hematoxyline according to the conventional method or with Ehrlich's hematoxyline, and drawings were made in the clear chamber.

Female gametophyte development and outset of embryo differentiation in members of the family Vitaceae

The embryology of the Vitaceae roused the interest of investigators since some time ago. Thus, for instance, Bioletti (1921), Ivanova (1928), Pearson (1923), Sydnes (1935), and Stout (1936, cited in Mulay, Nair and Sastry, 1953) carried out studies in seedless 'vines'; Baranov (1946), in preparation of the 'Ampelography of the U.S.S.R.', investigated the macrosporogenesis, the fecundation process and the embryo formation in *Vitis vinifera* and in some of its Asian cultivated forms; Mulay, Nair and Sastry (1953) studied the male and female gametophyte development in *Vitis pedata*; Nair and Bajaj published in 1966 a paper on the floral morphology and the embryology of *Cyphostemma setosum* (Wall.) Alston.

In Rumania, Dvornic (1961) made some observations concerning the fecundation process and the female gametophyte development in the

variety 'Hamburg Muscatel', though not based upon direct experimental investigation.

This research line is far from including members from all the genera of this large family.

The existing gaps in the embryological knowledge of the family Vitaceae determined us to include in our investigations the female gametophyte development, the fecundation process, the embryo and the secondary endosperm formation in 12 taxonomic units, namely 10 species belonging to 8 genera and 2 cultivated forms. Among these, a *Leea* species was studied, a genus often ascribed to the Vitaceae, though, according to the current opinion, building up an independent family within the Rhamnales Order. Embryological data on members of the Rhamnaceae family from the same order are found in the extensive work of Tullio (1947).

The 12 taxonomic units investigated by us are the following :

a) Family VITACEAE : *Vitis silvestris* Gmel. ; grapevine varieties 'Pinot gris' and 'Gordin' ; *Parthenocissus quinquefolia* (L.) Planch. ; *Ampelopsis heterophylla* Blume ; *Cissus assamica* (Laws.) Craib. ; *Cissus discolor* Bl. ; *Cissus cactiformis* Gilg. ; *Cayratia pedata* (Lam.) Juss. ; *Tetrastigma lanceolarium* Pl. ; *Ampelocissus thyrsoflora* (Bl.) Planch. ;

b) Family LEEACEAE : *Leea rubra* Bl.

A description of the morphological peculiarities of the female gametophyte development in the 12 taxonomic units mentioned above is given hereunder.

Vitis silvestris Gmel. (Botanical Garden in Bucharest). Plates I-V, figures 1—21.

This species is spread in the Danube countries. It is the only spontaneous *Vitis* species in Rumania. It displays greenishyellow, polygamous, almost perfectly dioecious flowers standing on dimorphous plant individuals. The flowers are small, of the type 5, with 5 stamens. The female flowers display short-filamented stamens bearing sterile pollen, while the male ones have stamen filaments exceeding the length of the petals ; the gynoeceum is coenocarpic-eusyncarpic, built by the concrescence of two carpels ; it has two ovarian nidi, each nidus sheltering at the bottom part of the septum two anatropic, apotropic, ascending, bitegumentate, crassinucellate ovules. The longitudinal section shows the ovarian cavity widens towards the style and has the aspect of an elongated triangle, while the rest of it, around the ovule, is of restricted volume. There initially occurs a prominence built up by uniform cells (figs 1, 2), of slanting growth : it is the ovule primordium. Concurrently with the beginning differentiation of the inner integument (ovule envelope), the primordium starts to bend sideways in the cavity of the ovarian nidus (figs 3, 4). On the prominence top, in the sub-epidermal layer of the young nucellus, a big plasma-rich cell with a large nucleus is distinctly seen. This cell divides periclinally forming outwards a parietal cell, which, by repeated periclinal and anticlinal divisions, will generate in a fan-shaped arrangement 5—7 cell layers constituting the nucellar calotte, while inwards a cell which is the macrosporal (megasporal) mother cell (figs 2, 4, 6). Concurrently with the formation of the nucellar

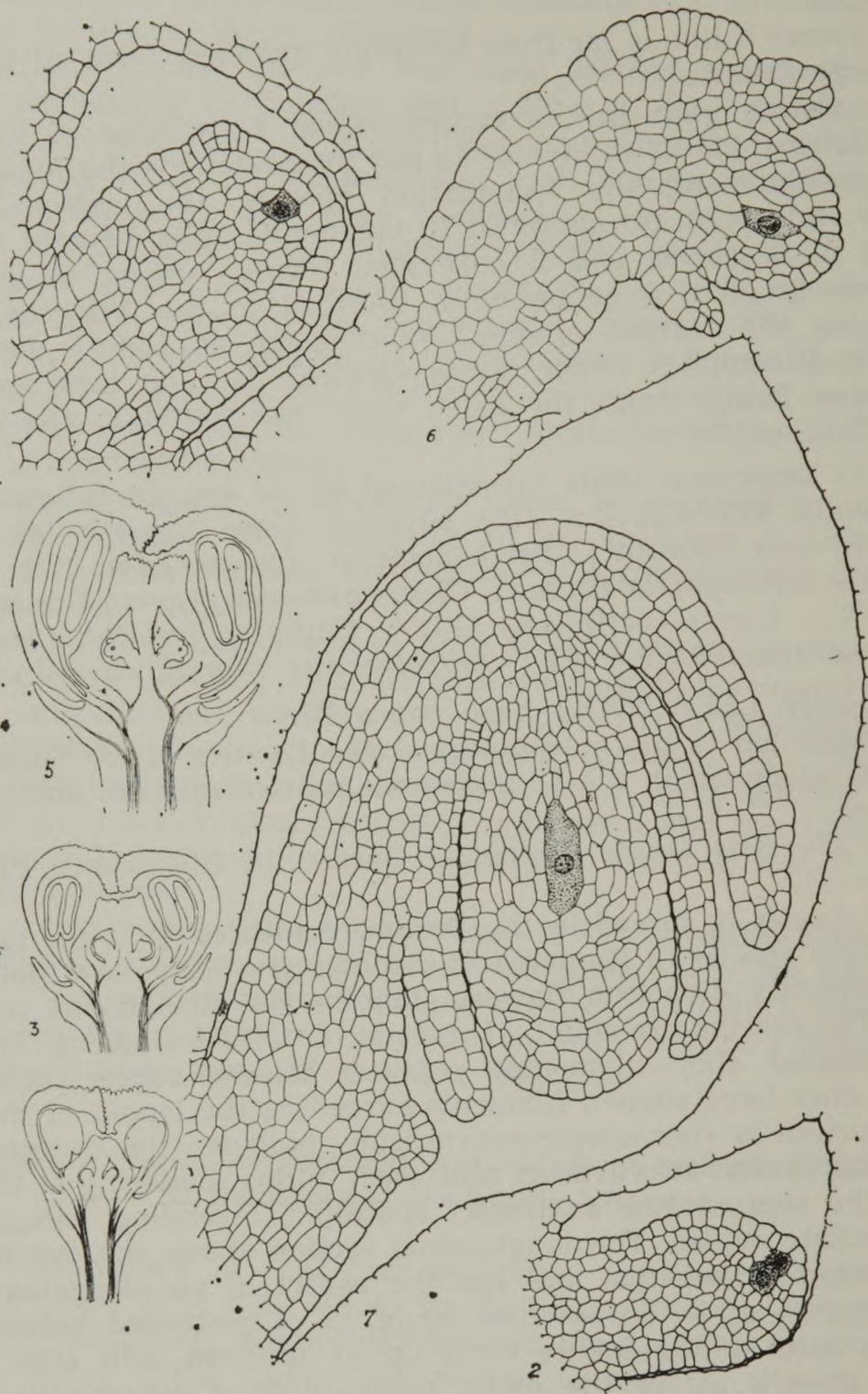


PLATE I

Vitis silvestris Gmel.

Text — fig. 1—7, flower and ovule in longisection in divers stages of development. 1—2, young ovule showing macrosporal mother cell and parietal cell ; 3—6, young ovule with macrosporal mother cell situated appically, the inner integument starts growing ; 7, the partially development of the nucellar and epidermal calottes, the macrosporal mother cell is in the centre of the nucella, the integument are developed.

Drawing 1, 3, 5, = 50 X ; 2, 4, 6, 7 = 440 X. Original.

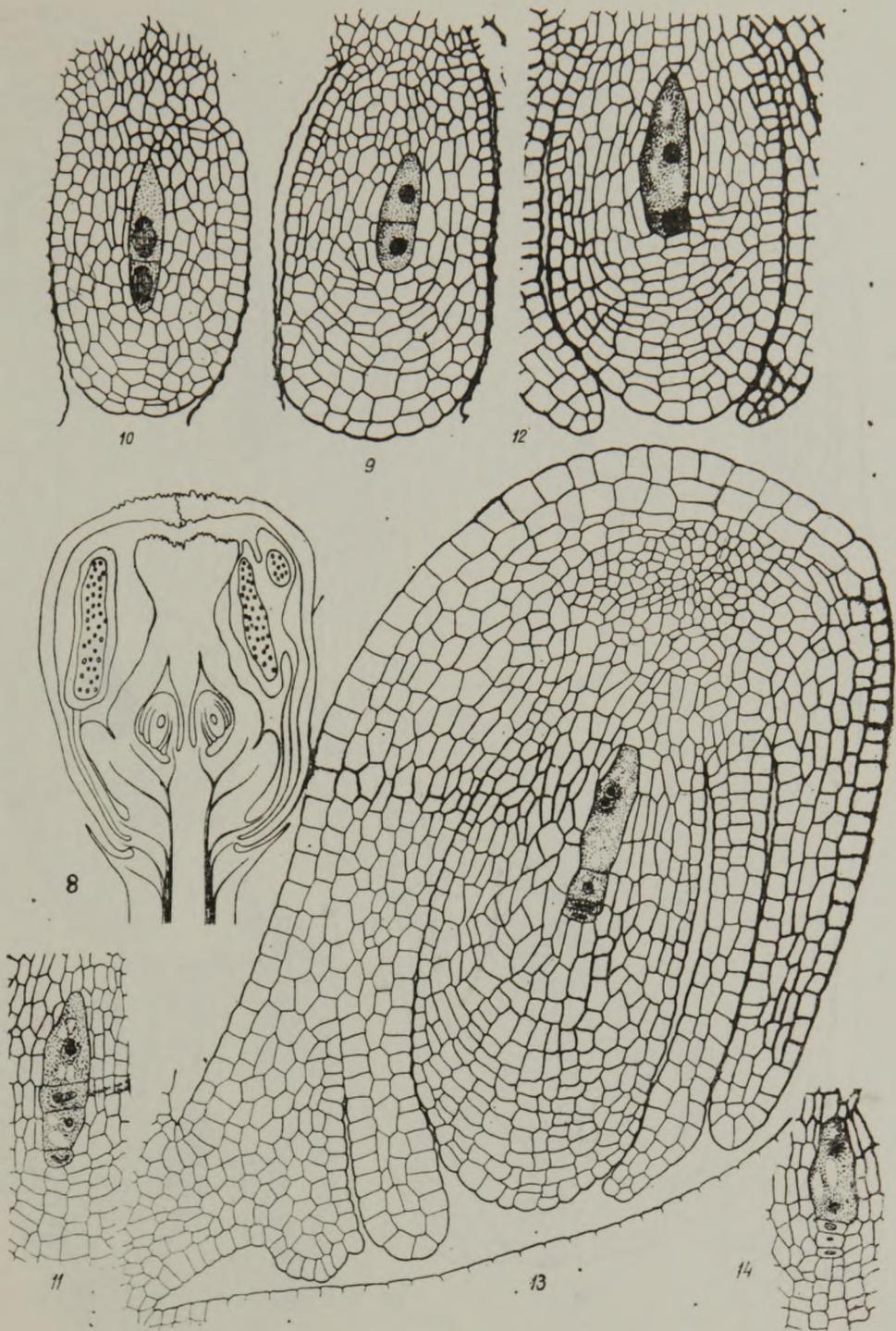


PLATE II

Vitis silvestris Gmel.

Text — fig. 8—14, flower and ovule in longisection ; 8, flower in longisection, in the pollinic sacs the pollen grains are complete developed ; 9, in the centre of the nucella the first division of the macrosporal mother cell ; 10, achievement of the uniseriate tetrad of macrospores ; 11—12, a linear tetrad of macrospores, with the fourth macrospore functioning ; 13—14, the beginning of the differentiation of the embryo-sac.

Drawing 8 = 50 \times ; 9—14 = 440 \times . Original.

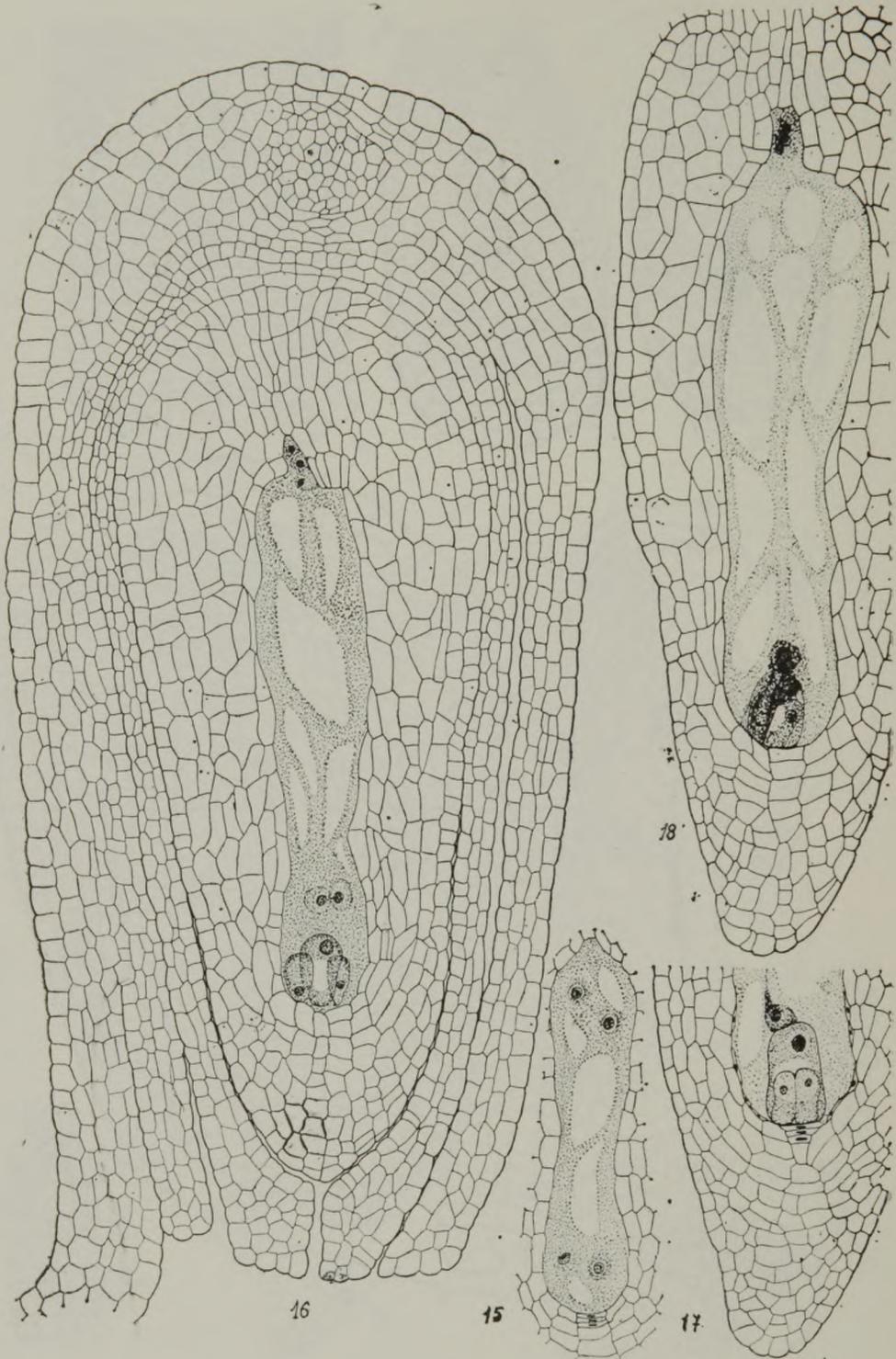


PLATE III

Vitis silvestris Gmel.

Text — fig. 15—18, ovule in longisection, 15, four-nucleate stage of the development of the embryo-sac; 16, the ovule complete formed, the micropyle is delimited by the inner integument only, the 8-nucleate Polygonum-type embryo-sac, before fusion of the polar nuclei; 17, the micropylar area of the embryo-sac with the oosphere apparatus and the secondary nucleus adhering to the oosphere; 18, the embryo-sac in the process of double fecundation.

Drawing 440. X. Original.

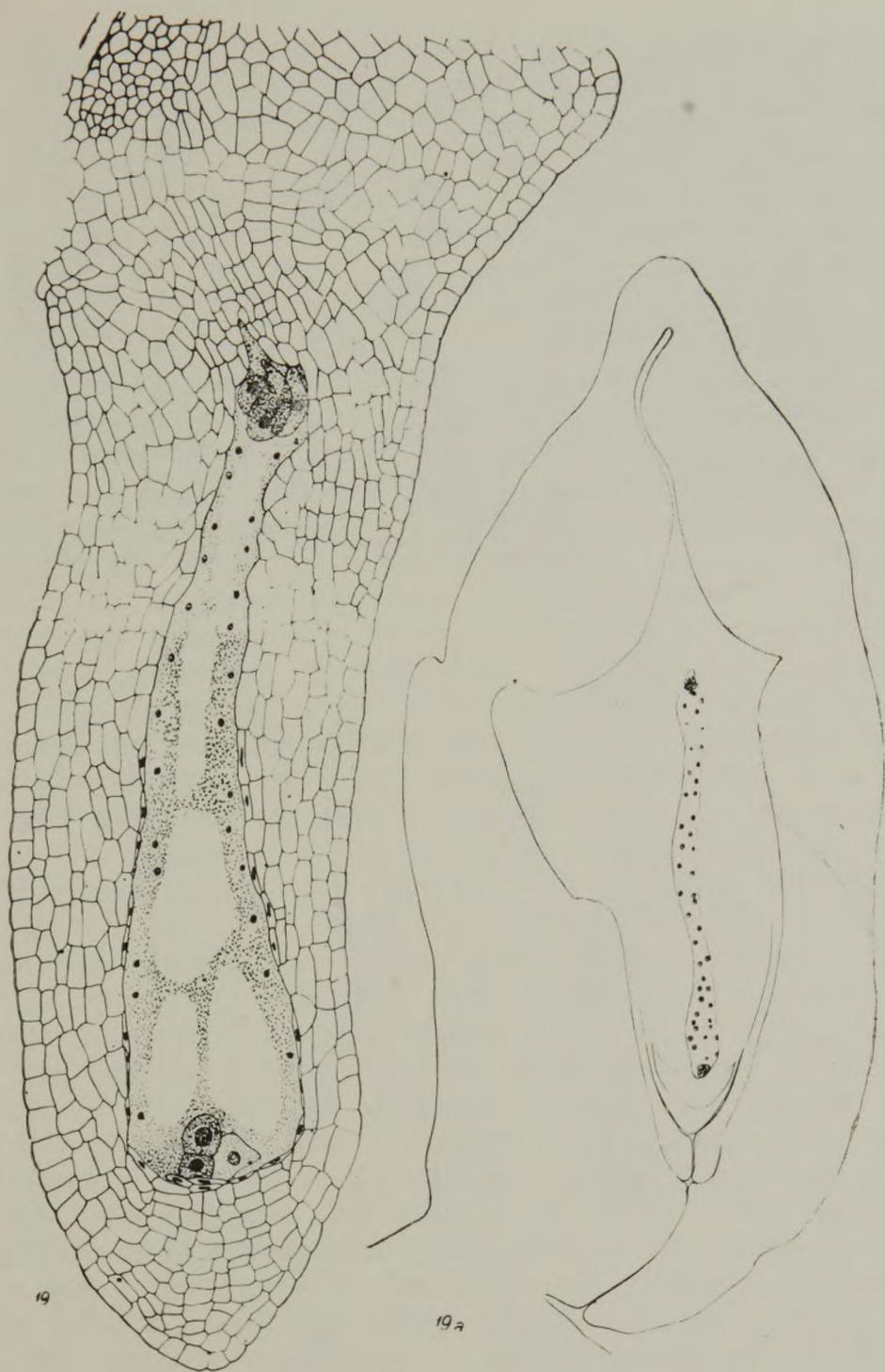


PLATE IV

Vitis silvestris Gmel.

Text — fig. 19—19-a, ovule in longisection. 19, stage of the nuclear-type endosperm and a bicellular pro-embryo; one of the synergids survives, the antipodes are \pm big and a pyriform shape: 19a, the ovule in longisection, the outer integument has a considerable growth, surpassing the inner one and covering the inner integument's micropyle.

Drawing 19 = 440 \times : 19a = 50 \times . Original.

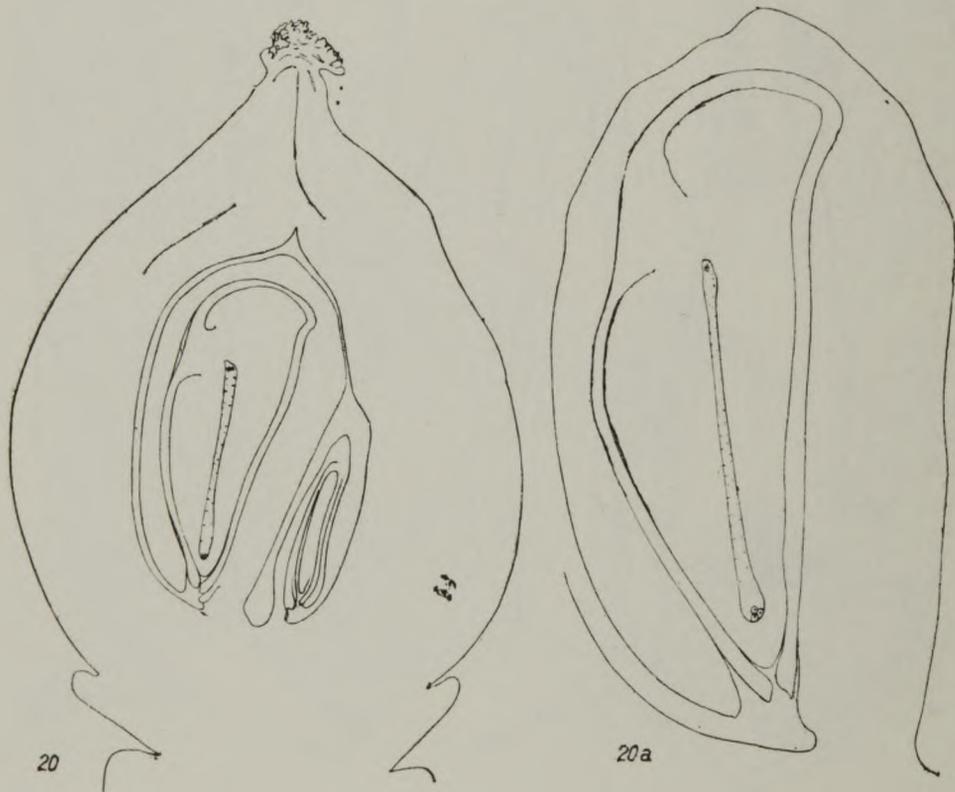
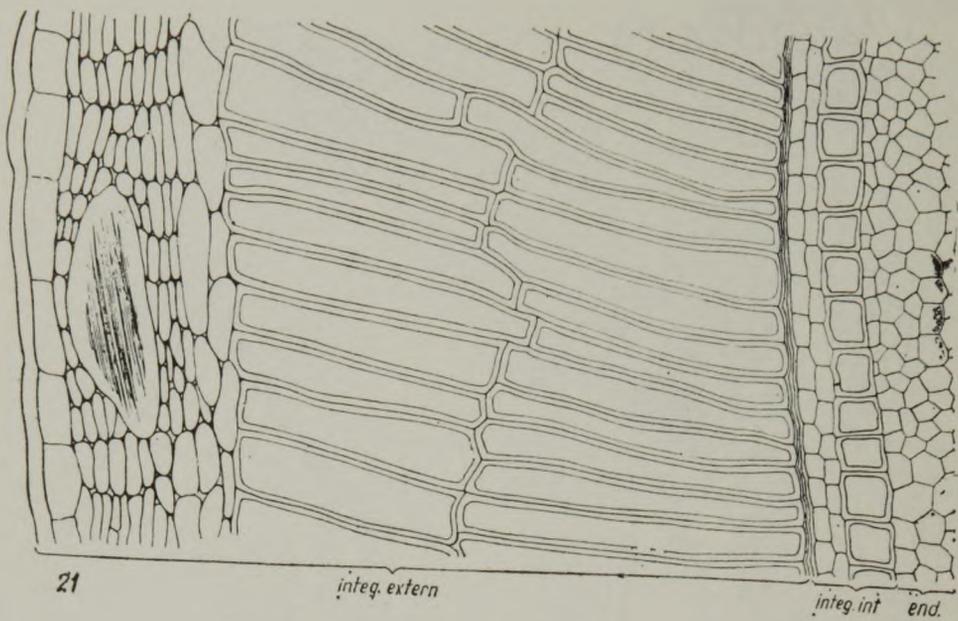


PLATE V

Vitis silvestris Gmel.

Text — fig. 20—21. 20, ovule in longisection, after the proces of fecundation ; 20a, the ovule transformations after the double fecundation, the chalaza passing over to the ventral size ; 21, part of the seed anvelope, increased.
 Drawing 20 = 50 X ; 21 = 440 X. Original.

calotte, 3—5 cell layers constituting the epidermal calotte come into being from the epidermal cells of the nucellar apex by periclinal divisions.

Due to the occurrence and development of the two calottes (the nucellar and the epidermal one) the macrosporal mother cell will gradually get a deeper location, eventually occupying the centre of the nucellus; as a rule, this cell is broader towards the micropyle and narrower towards the chalaza (figs 7). Meanwhile, the ovule increases in volume, its inner integument appears and starts growing, not covering, however, the nucellus, while, opposite to the funicle (ovule stalk), the outer integument almost catches up with the inner one, in contrast with the ovule stalk area, where it is merely sketched (figs 12, 13).

At this stage, the macrosporal mother cell divides by meiosis in two cells, then it divides equationally, and a uniserial tetrad of macrospores is achieved (figs. 8, 9, 10). The three apical macrospores will then disorganize, beginning with the first and the third one, then the second one or, alternatively, the first two will start, followed later by the third one (figs 11, 13), while the chalazal one will continue developing and his nucleus, by repeated divisions, will bring forth the eight-nucleate Polygonum-type embryo sac. At the eight-nucleate stage of the embryo sac the ovule formation is completed; the nucellus displays its two calottes fully developed; the inner integument formed by 3—4 cell layers covers the nucellus apically delimiting the micropyle, while opposite to the ovule stalk the outer integument formed by 3—4 cell layers may or may not come up with in growth the inner one, without participating in the formation of the micropyle; towards the ovule stalk it is poorly developed, being built together with the ovule stalk by 6—7 cell layers (figs 13, 14, 15). During the differentiation of the embryo sac from the eight nuclei, the ovule grows mainly through the volume increase of the cells, while the size growth of the embryo sac also happens by the consumption of a part of the nucellus and almost entirely of the nucellar calotte (fig. 16). From the eight nuclei there will be formed: — the oosphere apparatus, sited in the broader micropylar area of the embryo sac and including the oosphere, with the nucleus at the bottom, a vacuole over it and two synergids, devoid of vacuoles, smaller than the oosphere and sited on its sides; — the antipodal apparatus built by three superposed nuclei sited in the embryo sac's chalazal area, the latter narrowing in the shape of haustorial extensions; the two polar nuclei found in a dense plasma bag are making their way towards the oosphere apparatus, in the proximity of which they will merge together building the secondary nucleus of the embryo sac (figs 16, 17). At the time the pollen grains set out germinating on the stigma and the pollinic tubes start penetrating the style, the embryo sac's secondary nucleus adheres to the oosphere (fig. 17). At the same time with the full differentiation of the embryo sac its size will increase considerably. Such is the structural aspect of the embryo sac just before the fecundation process. Also, concurrently with the maturation of the embryo sac, the inner epidermis of the two integuments, particularly in their micropylar portion, gets a brownish colour due to the nutrients

accumulated there, these substances playing a role both in the attraction and the feeding of pollinic tubes.

Baranov (1946) reported that antipodes in *Vitis silvestris* and in *V. vinifera* get disorganized before the fecundation process, a fact which could not be confirmed in the material investigated by us.

The pollinic tube penetrates through the micropyle (porogamia), the gametes, carrying a large amount of plasma, penetrate in one of the synergids, one gamete joining the oosphere nucleus, while the other joins the embryo sac's secondary nucleus adhering to the oosphere. The fusion of the four nuclei in the process of double fecundation happens more or less simultaneously (fig. 18).

In *Vitis silvestris* the fecundation process takes place shortly upon the pollenization. In the analysis of artificially pollenized gynoecea, sampled and fixed every 15 minutes, we met the fecundation phenomenon 60 minutes after the pollenization. In the available literature we did not find any specific statement as to the duration of the fecundation in members of the family Vitaceae, except for Baranov's remark (1946) that "the effects of fecundation in the 'grapevine' can be observed the day after pollenization, as materialized in the perceptible increase of the ovary".

During the fecundation process the antipodes slightly increase their volume, get coated by an obvious plasmatic film and have a more or less pyriform shape (fig. 18). The haustorial extension of the embryo sac containing the antipodes shifts towards the area opposite to the ovule stalk, while the nucellus cells at the embryo sac basis become prolonged in a fan-shaped manner towards chalaza (the conductive tissue), which gets a ventrally-basal position through the ovule transformations.

As a result of the double fecundation the zygote and the accessory zygote come into being, the latter shifts towards the embryo sac centre and very soon starts to divide; however, these divisions are not followed by an occurrence of dividing membranes, but a nuclear-type endosperm arises, developing at the expense of the nucellus, that is consuming its cells around the embryo sac. Meanwhile, the zygote undergoes its first transversal partition, thereby forming a two-cell pro-embryo; the synergid penetrated by the pollinic tube gets disorganized, while the other survives, its nucleus assuming a more or less basal position (fig. 19).

Concurrently with these transformations occurring in the embryo sac and in parallel to the beginning disorganization of nucellar cells around the embryo sac (where, at this stage, a nuclear endosperm and a bicellular pro-embryo are found), numerous alterations occur in the ovule body, as well as in its wall. Out of the four ovules present in the ovary, only one (rarely two) will grow to become a seed, while in the others the embryo sacs will degenerate: a very common phenomenon in the Vitaceae.

The integuments also undergo significant transformations, namely: the outer ovule envelope has a considerable growth, surpassing the inner one and eventually covering the inner integument's micropyle (fig. 19 a);

the exterior epidermis of the ovule envelope subsists under the aspect of a layer of tangentially flattened cells; the middle cell layer multiplies, its cells increase in size and accumulate an appreciable amount of calcium oxalate in the shape of raphids, and they finally become giant cells; the interior epidermis of this integument divides, giving rise to several regularly arrayed cell layers, the number of cell rows differing in the various seed areas (being most numerous in the micropyle area), so that, in the end, these much elongated cells, bearing thickened membranes, offer a solid coat to the seed. The inner integument undergoes less alteration: both the exterior epidermis and the middle cell layer remain unchanged; the interior epidermis, in the seed maturation period, gets its membranes thickened and brown-coloured, therefore this cell layer neatly delimits the secondary endosperm gradually building up out of the nuclear endosperm (starting from the basis of the embryo sac and eventually surrounding the embryo formed in the micropyle area). The complete formation of the embryo and of the secondary endosperm coincides with the total disintegration of the nucellus, so that in the mature seed no nucellar remains are found (fig. 21).

These transformations are accompanied by a slight bending of the ovule, the chalaza passing over to the ventral side and the embryo sac basal part incurving towards the chalaza (figs 20, 20 a).

The ovule alterations during the seed formation process are coexisting further with a massive growth of the ovary wall and with a necrosis process of the stigma and of the style (figs 20, 21).

The cultivated form "*Pinot gris*" (Ampelographic Collection of the Agronomical Institute in Bucharest). Plates VI-VII, figures 1—4.

It is originating from French, autofertil, spread in majority vineyard in Europa; in Rumania is cultivated in the biggest vineyard. It is a cosmopolite sort. It present bisexual flowers, normals, pentamerous, bicarpellar, bilocular gynoecium, with 2 ovules in each locule. Ovary pyriform with very small stil and a dilatated stigmat.

The ovule is anatropous-apatropous ascendent, bitegmic and crassinucellate (placed in a restricted ovarian cavity), similar to that of *Vitis silvestris*, from which is differs in: the outer integument consisting of 5—6 cellular layers; the epidermal and nucellar callotes are more developed (from 6—7 or respectively from 10—15 cellular layers), but which are consumed to the development of the embryo-sac.

The macrosporal cell is placed in the middle of the nucella, because the callotes (nucellar and epidermal) are more developed, it is prolonged and slender (fig. 1).

The embryo sac who is formed by repeated division of the chalazal macrospore, is similar to that of *Vitis silvestris* from which is differs in that it is usually alike wide in all its length, or more wide at the base above of the haustorial extensions, the two polar nuclei merge together building the secondary nucleus in the central part of the embryo sac and after that it is making its way towards the oosphere apparatus; the antipods are in the haustorial projection triangulary disposed; the synergids have distintly basal vacuoles. Similar to that of *Vitis silvestris*

the inner epidermis of the two integuments gets a brownish colour due to the nutrients accumulated there.

The fecundation proces took in 15 minutes after pollenization and it is similar to that of *Vitis silvestris*, from which it differs in that one antipod undergoes resorbtion while the remaining two ones increase in volume, become wrapped into a plasmatic film, they are pyriform and they have the distinctly basal vacuoles (figs 2, 3).

Unlike *Vitis silvestris*, at this cultivated form, in the nuclear endosperm stage, when the pro-embryo is bi or-tricellular, the outer integument grows very much in the micropylar area, adhere closely and fuse together closed the micropyle (fig. 4).

The other transformations for produce the seed are similarly as in *Vitis silvestris*.

The cultivated form "*Gordin*" (Ampelographic Collection of the Agronomical Institute in Bucharest). Plate VII, figures 5—7.

It is one of the oldest rumanian cultivated form, cultivated in our vineyard only, closed fertilization. Bisexual flowers, pentamerous, bicarpellar, bilocular gynoecium, 2 ovules developing in each locule. The longitudinal section shows a trapezoidal cavity towards the style, while the rest of it, around the ovule is of restricted volume. The ovary has a pyriform shape, a very short style and a dilatated stigmatate.

The ovule is anatropous-apotropous, ascendant, bitegmic and crassinucellate, the micropyle is delimitedated by the inner integument only. There is a nucellare calotte formed by 6—7 cell layers and an epidermal one formed by 6—7 cell layers, too.

The development of the ovule, the calottes and of the embryo sac is similar to that of *Vitis silvestris*; the embryo sac is eight-nucleate, by Polygonum type.

Unlike *Vitis silvestris*, to this cultivated form the embryo sac is very long and slender; the three antipods are situated one beneath the other in the haustorial projection of the embryo-sac (figs. 5—6). At the time of fecundation the antipods increase in volume, become wrapped into a plasmatic film, become more or less spherical in shape.

The proces of double fecundation do not happen simultaneously like in *Vitis silvestris* (fig. 7).

The forming of the envelope of seed is similar to that of *Vitis silvestris*.

Parthenocissus quinquefolia (L.) Planch. (Botanical Garden in Bucharest). Plates VIII-XI, figures 1—15. It is a robust creeper originating from North America. It is cultivated for its ornamental qualities, to cover walls. It present bisexual flowers: cuplike pentamerous calyx; pentamerous dialipetalous corolla; androceus consting in 5 stamens; bicarpellar, coencarpeous, bilocular gynoecium, 2 ovules developing in each locule. Ovary pyriform, having very short style and reduced stigmatate. The ovules develop at the carpellar base. They are anatropous-apotropous ascendent, bitegmic and crassinucellate. In initial stages of development of the ovules the ovarian cavity is triangular in shape toward the style and in the micropylar area. When ovules are fully

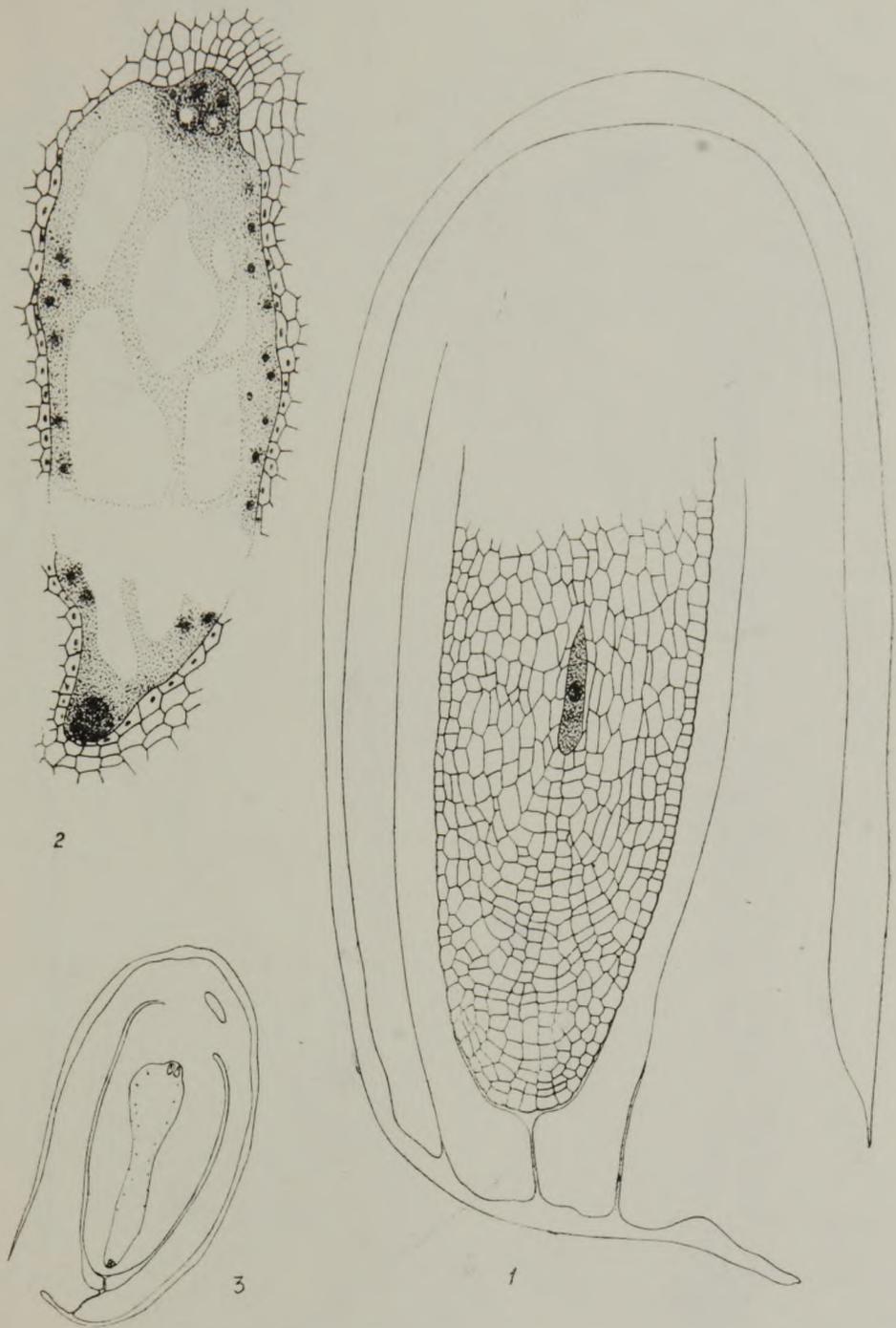


PLATE VI

The cultivated form „Pinot gris“

Text — fig. 1—3, ovule in longisection. 1, ovule complete formed, the micropyle is delimited by the inner integument only, the epidermal and nucellar calottes are well developed, the macrosporal mother cell is placed in the middle of the nucella; 2, bicellular pro-embryo stage and the nuclear-type endosperm; one of the antipodes resorbed, while the remaining two ones increase in volume, are wrapped into a plasmatic film, they are pyriform shape and distinctly basal vacuoles; 3, ovule in longisection with bicellular pro-embryo and the nuclear-type endosperm, the chalaza passing over to the ventral side, the micropyle is covering by the considerable growth of the outer integument.

Drawing 1,2 = 440 X. Original.

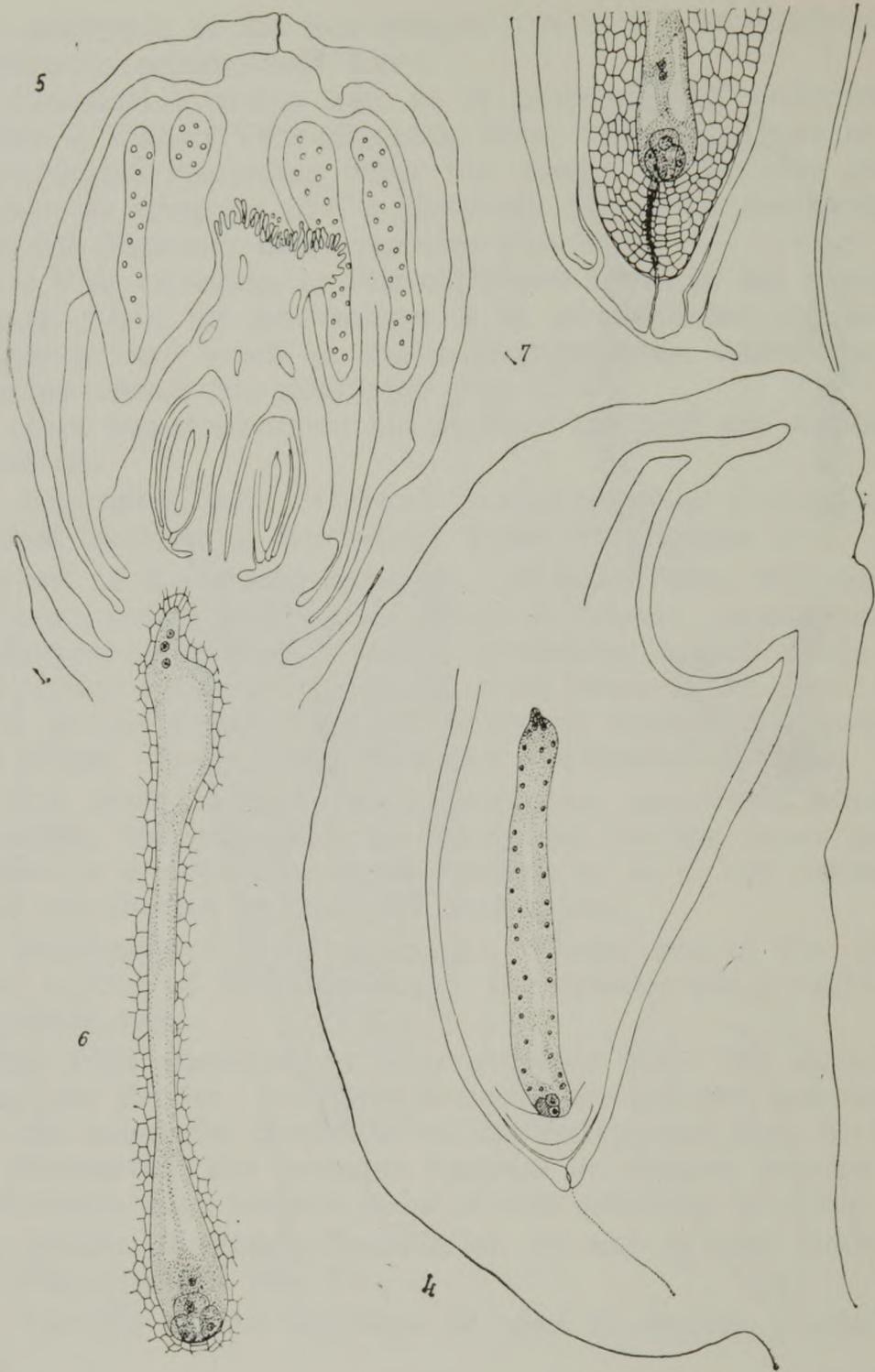


PLATE VII

The cultivated form „Pinot gris“

Text — fig. 4, ovule in longisection, embryo-sac with bicellular pro-embryo and the nuclear-type endosperm.

The cultivated form „Gordin“

Text — fig. 5—7, ovule and flower in longisection. 5, flower in longisection, eight-nucleate embryo-sac, in the pollinic sacs the pollen grains are complete developed; 6, mature embryo-sac, long and slender, the antipodes are situated one beneath the other in the haustorial projection of the embryo-sac; 7, the embryo-sac after the fecundation.

Drawing 5 = 50 X; 6, 7 = 240 X. Original.

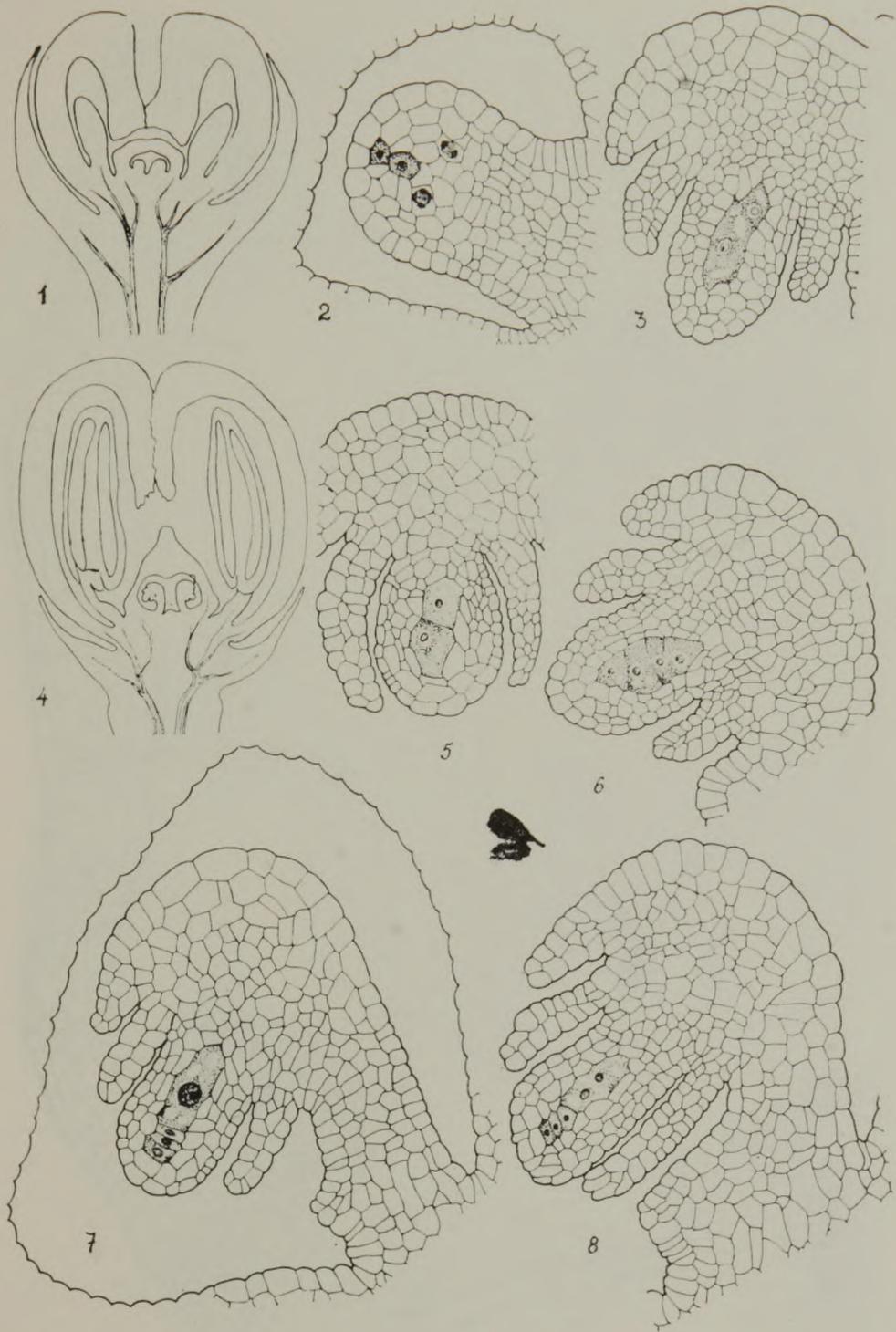


PLATE VIII .

Parthenocissus quinquefolia (L.) Planch.

Text — fig. 1—8, ovule and flower in longisection. 1—5, young ovule in longisection showing macrosporal mother cell; 6, nucella in longisection to show linear tetrad of macrospores; 7, a linear tetrad with the fourth macrospore functioning; 8, two nucleate female gametophyte.

Drawing 1, 4 = 50 X; 2, 3, 5—8 = 440 X. Original.

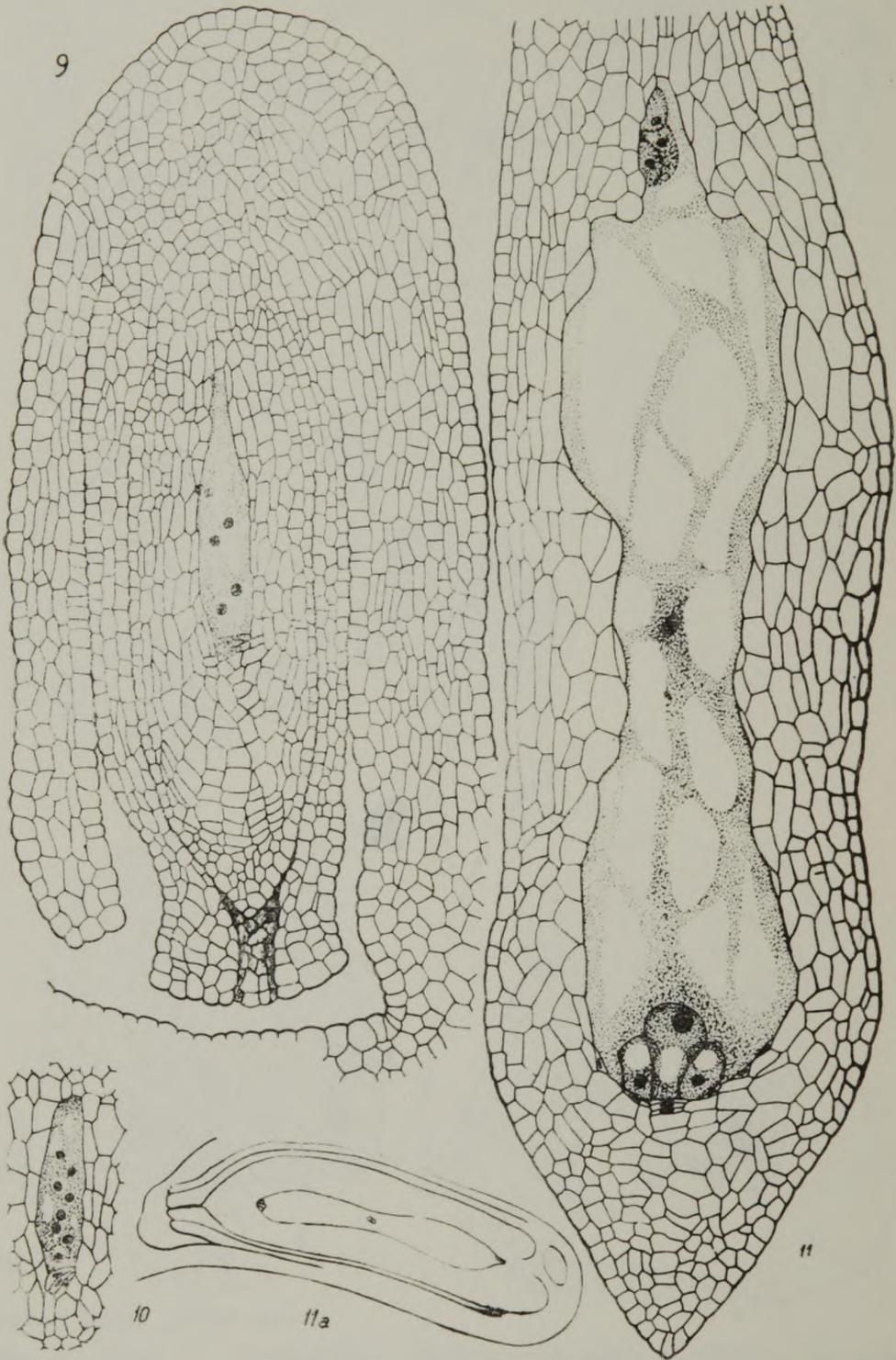


PLATE IX

Parthenocissus quinquefolia (L.) Planch.

Text — fig. 9—11, ovule in longisection, development of ovule and embryo-sac, Embryo-sac before fusion of polar nuclei; 11a, embryo-sac developed.

Drawing = 440 \times . Original.

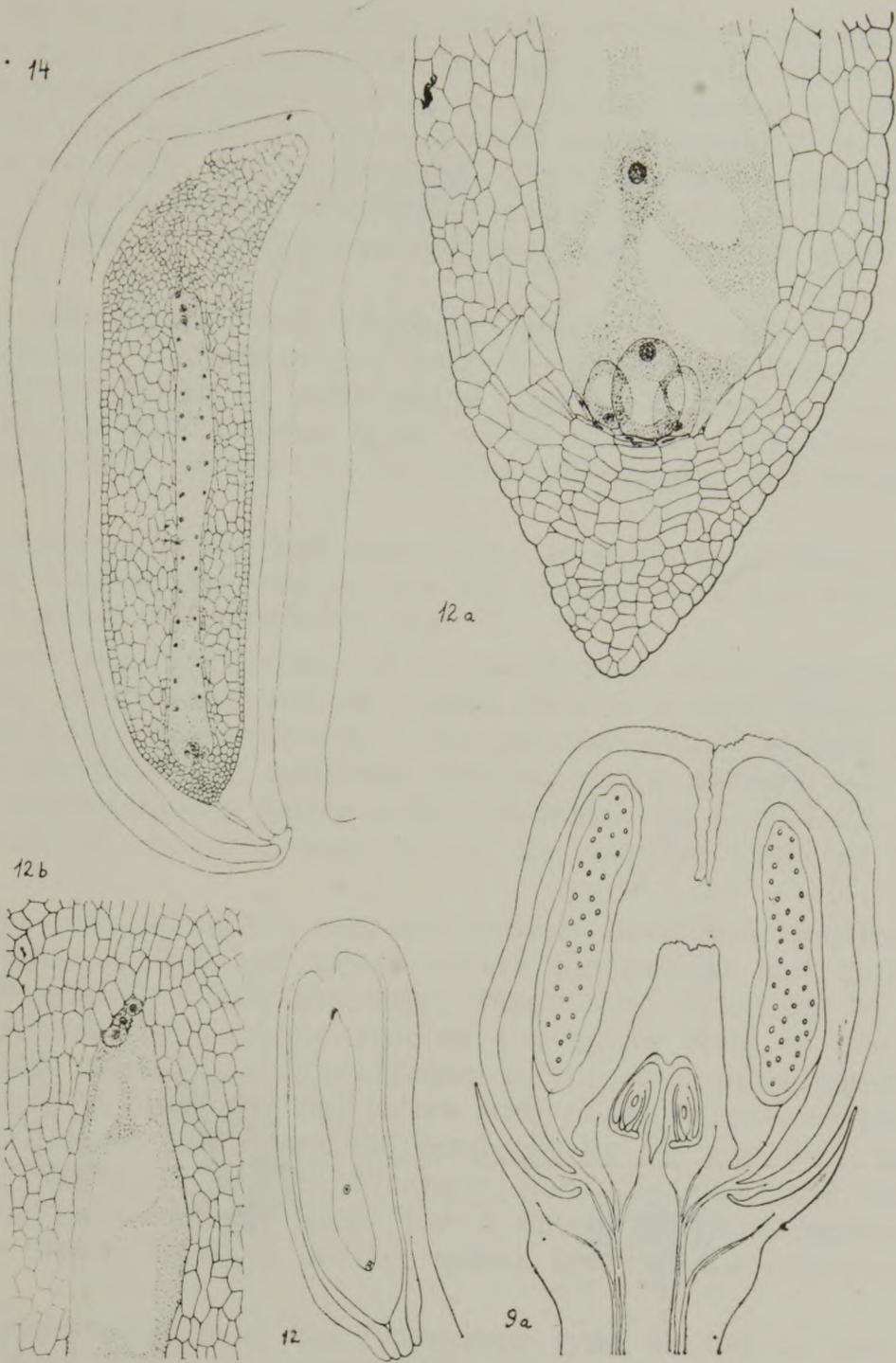


PLATE X

Parthenocissus quinquefolia (L.) Planch.

Text — fig. 9a, 12a, b, 14, flower and ovule in longisection. Mature embryo-sac; 14, stages in development of embryo (bi-cell proembryo); stages in the development of endosperm.

Drawing 9a = 50 X; 12 = 440 X. Original.

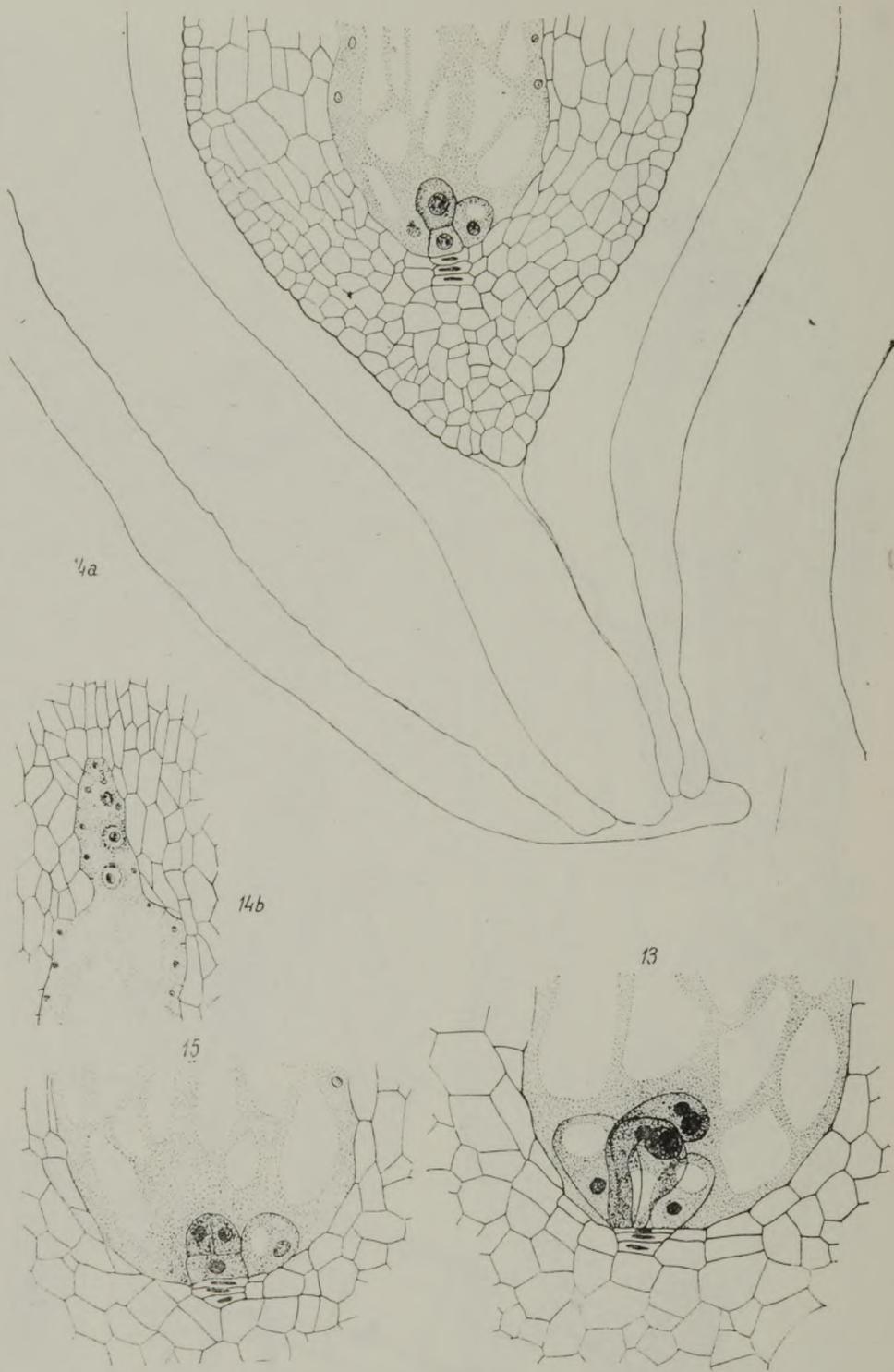


PLATE XI

Parthenocissus quinquefolia (L.) Planch.

Text — fig. 13, 14a, b, 15, ovule in longisection. 13, 15, simultaneous fertilization, stages in the development of nuclear endosperm, the nucella is stiped and the endosperm cross hatched; 13, tri-cells pro-embryo.
 Drawing 13 = 550 X; 14, 15 = 440 X. Original.

developed the ovarian cavity becomes reduced as a narrow space surrounding the ovules.

At the beginning, a uniform cellular prominence appears from the carpellar base in the ovarian cavity, representing the primordial ovule. While growing, the latter undergoes a lateral curving in the ovarian cavity. At the top of the prominence, subepidermally, in the young nucella a large cell, rich in plasma and having a large nucleus is distinguished. This undergoes periclinal division to produce a parietal cell outwardly and a macrosporal (megasporal) mother cell inwardly (figs. 1, 2).

The macrosporal cell increases rapidly in mass and undergoes meiotic division to produce two cells, then again equational division to obtain a linear tetrad of macrospores (figs 3—6). During this time the nucella hardly differentiates at all while the inner integument and the outer integument opposite the funicular side appear and become developed.

The nucleus of the last macrospore soon starts dividing while the remaining 3 apical macrospores are still distinguished and the caps have not yet started differentiation (fig. 7).

Between the first division of the fourth macrospore (now the mother cell of the embryo-sac) and its second division a rapid development of the ovule body occurs, namely: the nucellar cap is formed following repeated, periclinal and anticlinal division of the parietal cell, made of 10 to 12 cellular layers, and also the epidermal cap as a result of repeated division of one epidermal cell of the nucella, made of 5—7 cell layers.

At the time when the caps are entirely developed, the inner integument made of 3(4) cell layers covers the nucella and delimits the micropyle apically while the outer integument, consisting of 5(6) cell layers opposite the funicule reaches the apical level of the nucella. At this stage there is no differentiation of the outer integument towards the funicule. At this stage, the future embryo-sac is four-nucleate, situated in the middle of the nucella, protected by the two caps, almost of the same width all along its length, suddenly narrowed chalazally. During the eight-nucleate stage the 2 micropylar macrospores appear still very much flattened in the apical area of the embryo-sac (figs 8—10).

The embryo-sac is of the Polygonum type, similar to that of *Vitis silvestris* from which it differs in that it is wider at the level of the egg apparatus, much more narrowed in the middle where two polar nuclei are fused, then it becomes again wider and terminates in a more or less deep narrow haustorial projection (figs. 11, 11 a). On one side of the haustorial projection there are 3 laterally occurring antipods (fig. 12 b). The secondary nucleus of the embryo-sac (which is formed at the center of the sac) moves toward the oosphere to which it eventually becomes attached. The two gametes introduced through the pollinar tube which protrudes through the micropyle (porogamy) find the nucleus of the oosphere and the secondary nucleus of the embryo-sac, very close

to each other, and the fusion of the nuclei occurs almost simultaneously (fig. 13).

After fertilization is completed, the accessory zygote obtained from the fusion of the gamete with the secondary nucleus of the embryo-sac moves toward the base of the embryo-sac, its food source, where it initiates division to produce a secondary endosperm of the nuclear type. During this time one antipod undergoes resorption while the remaining two ones increase in mass, become wrapped into a plasmatic film, become more or less spherical in shape and are situated one beneath the other in the haustorial projection of the embryo-sac (figs. 14, 14 b).

The tissue surrounding the haustorial projection and especially the tissue at the base of it consists of elongate fan-like disposed cells having slightly thickened membranes. During the development of the embryo-sac and fertilization, the number of cell layers forming the nucella decreases as they are being eaten up by the growing embryo-sac.

After the nuclear endosperm is partly formed, the zygote undergoes, its first division to produce a bicellular pro-embryo (figs 14, 14 a).

Another feature of the ovules in *Parthenocissus quinquefolia* that distinguishes them from those in *Vitis silvestris* is that the micropylar area of the integuments increased notably following fertilization and become elongate. Both the inner and outer integuments, initially equal in length, adhere closely to the funiculus (fig. 14 a). It is only after the nuclear endosperm becomes developed in the embryo-sac and the pro-embryo reaches the bicellular stage that the outer integument opposite the funicular side overreaches in growth the inner integument to close the micropyle which following adhering of the inner integument becomes reduced to a very fine channel, obliquely to the nucella (fig. 14).

Simultaneous with these alterations that occur in the embryo-sac and in the micropylar area of the ovule, the cell layers of integuments undergo differentiation to produce the seed tegumen similarly as in *Vitis silvestris*. But unlike in the latter species, the chalaza moves in the present species slightly ventrally while remaining basal, a position that induces a very slight curving of the terminal part of the embryo-sac as well as of the fan-like tissue at its base (fig. 14).

Ampelopsis heterophylla Blume. (Botanical Garden of Bucharest). Plates XII—XIV, figures 1—11. It is a plant originating from Asia and North America. It presents bisexual flowers pentamerous. Bicarpellar, coenocarpeous, bilocular gynoecium, with 2 ovules in each locule. The ovules develop at the carpellar base, they are anatropous-apotropous, ascendant, bitegmic and crassinucellate. In initial stages of development of the ovule the ovarian cavity is \pm triangular towards the style. When ovules are fully developed the ovarian cavity became reduced as a narrow space surrounding the ovules. The stil is long and the stigmatic reduced (figs 1—3).

The ovules develop at the carpellar base, from which appears a uniform cellular prominence representing the primordial ovule. While growing, the latter undergoes on the beginning \pm horizontally, then curving in the ovarian cavity (figs 1—3).

The develop of the caps and the macroporal mother cell is similar to that of *Vitis silvestris*, from which it differs in : the caps are entirely

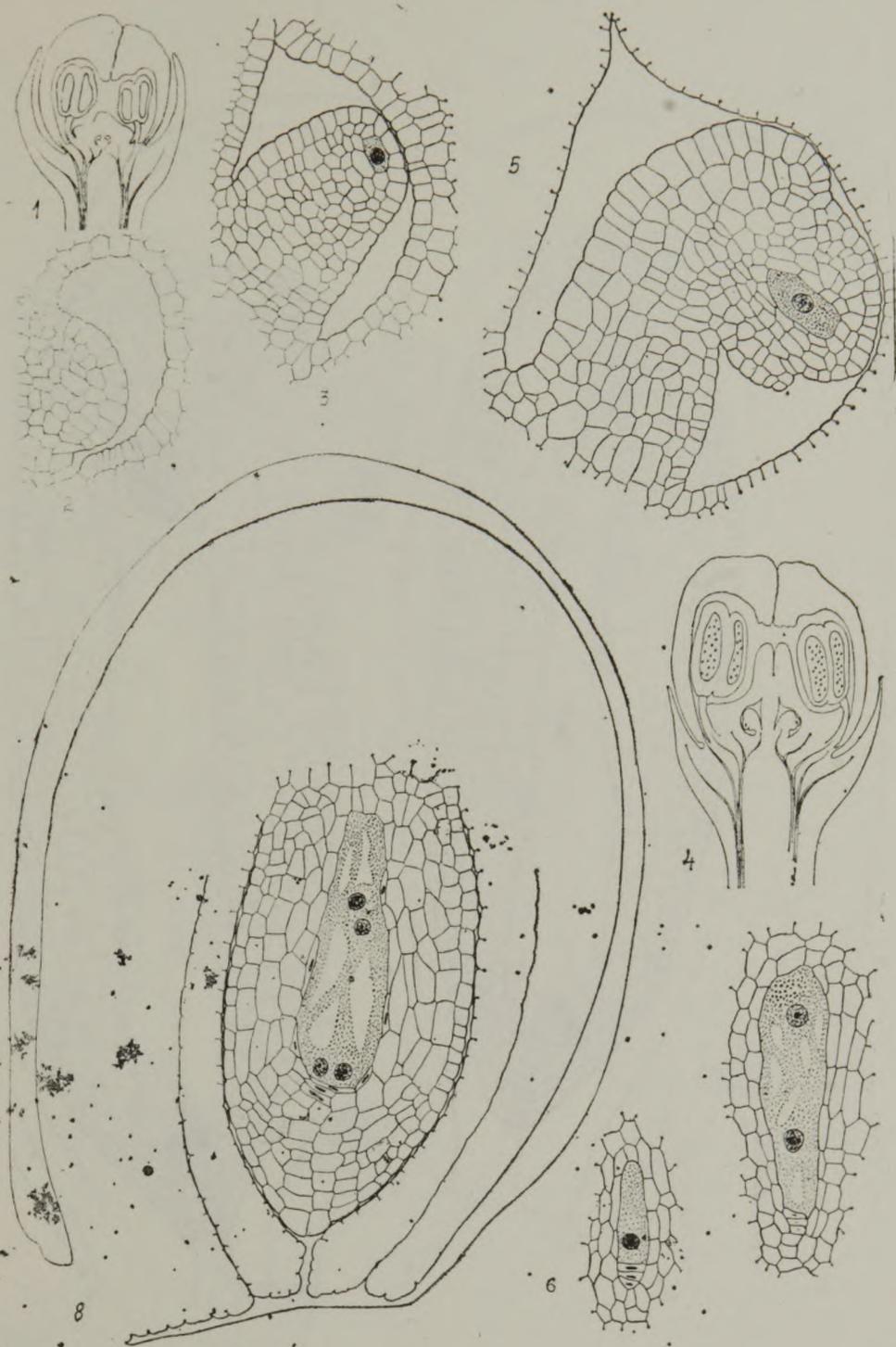


PLATE XII

Ampelopsis heterophylla Blume

Text — fig. 1—8, flower and ovule in longisection ; 1—2, young ovule formed by the uniform cells ; 3, young ovule with the macrosporal mother cell situated appically ; 4—5, beginning of the differentiation of the inner integument and a big macrosporal mother cell ; 6, a linear tetrad of macrospores with the fourth macrospore functioning (the mother cell of the embryo-sac) ; 7, 2-nucleate embryo-sac and the ovule completely formed, the epidermal and nucellar calottes fully developed, the micropyl is delimited by the inner integument only.

Drawing 1, 4 = 50 X ; 2, 3, 5—8 = 440 X. Original



9

10

PLATE XIII

Ampelopsis heterophylla Blume

Text — fig. 9—10, ovule in longisection. 9, 8-nucleate embryo-sac, the polar nuclei in the middle of the embryo-sac ; 10, the moment of the double fertilization, the pollinic tube penetrates through of the top of the integuments, the antipodes are big and they are pyriform shape.

Drawing 240 X. Original.

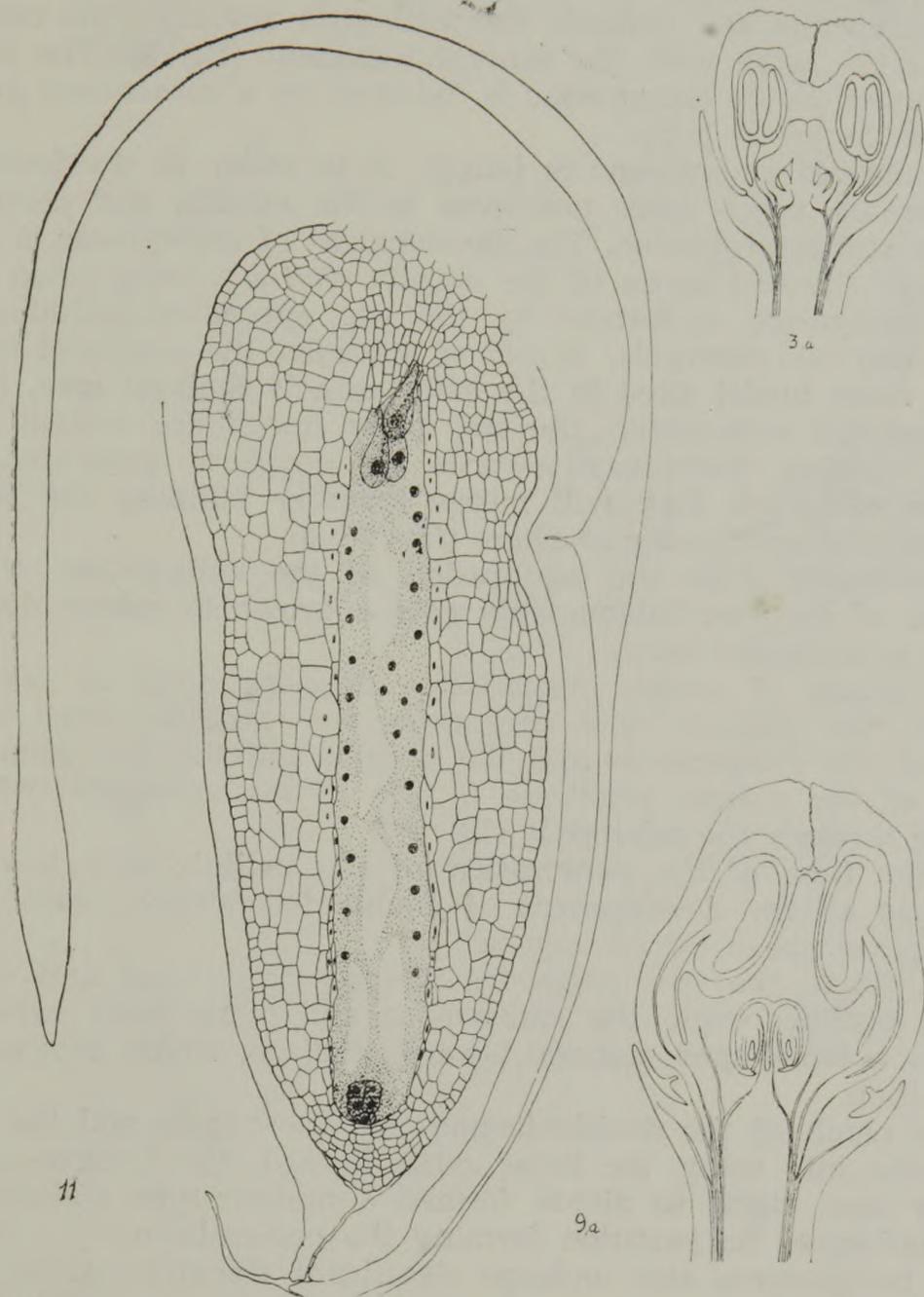


PLATE XIV

Ampelopsis heterophylla Blume

Text — fig. 3a, 9a, 11, flower and ovule in longisection. 3a, 9a, flower in longisection; 11, ovule in longisection after the double fecundation, the pro-embryo and the nuclear-type endosperm, the antipodes are big and pyriform, the outer integument surpassing the inner one and covering the inner integument's micropyle.

Drawing 3a, 9a = 50 X; 11 = 240 X Original.

developed while the young embryo-sac is in four-nucleate stage (figs 4—8). The caps are more reduced : the epidermal cap is formed of 4—5 cell layers, while the nucellar cap is formed of 2—3 cell layers only. In this stage the ovule is entirely developed, the inner integument covers the nucella and delimits the micropyle apically ; the outer integument is the same with the inner integument (fig. 8). The proces of differentiation of the embryo-sac is followed by a subsequent growth in the mass of the ovule body.

The mature embryo-sac is length, it is wider at the level of the egg apparatus, much more narrowed in the middle, and deep narrow of the haustorial projection. The development of embryo-sac is made by decreases of the cell layers of the nucella who are being eaten up. The mature embryo-sac is formed by the egg apparatus including a big oosphere and two synergids, devoid of vacuoles, the antipodal apparatus built by three nuclei sited in the embryo-sac's chalazal area, (a narrowing haustorial extensions), the two polar nuclei are central situated, then are making their way towards the oosphere apparatus, in the proximity of which they will merge together building the secondary nucleus who adheres to the oosphere (figs 9,9 a).

Concurrently with the maturation of the embryo-sac, the inner epidermis of the two integuments gets a brownish colour due to the nutrients accumulated there.

The proces of double fecundation happens more or less simultaneously, the pollinic tube penetrates the carpellar base an cross the top of the integuments and the nucella and the two gamets surrounded of the plasma penetrate in one of the synergids, without to penetrate through the micropyle (fig. 10).

At the time of the penetration of the pollinic tube towards the embryo-sac an the development of double fecundation takes place an increase in volume of the embryo-sac.

Also, found that the antipodes increase in volume and display a pyriform outline, while the embryo-sac and ovule basis become prolonged in a fan-shaped manner towards chalaza, which gets a ventral-basal position.

As a result of the double fecundation the zygote and the accesory zygote come into being, the latter shifts towards the embryo-sac centre and very soon starts to divide formed a nuclear-type endosperm, the zygote undergoes its partition forming the pro-embryo.

The integuments also undergo significant transformations namely : the outer ovule envelope has a considerable growth, surpassing the inner one and covering the inner integument's micropyle (fig. 19 a).

The forming of the envelope of seed is similar to that of *Vitis silverstris*.

Cissus assamica (Laws.) Craib. (= *Vitis assamica* Laws. ; = *Ampelopsis assamica* (?) ; *Vitis assamica* Fl.). (Botanical Garden in Bogor — Indonesia). Plates XV—XVI, figures 1—12.

It displays tetramerous flowers, with numerous simple unicellular and multicellular hairs on the flower stalk, on the outside of the calyx elements, on the outside of the corolla elements, as well as on the gynoeceum, particularly on the upper part of the ovary around the style



PLATE XV

Cissus assamica (Laws.) Craib.

Text — fig. 1—8, 12, flower and ovule in longisection. 1—2, young ovule with the macrosporal mother cell situated in the middle of the young nucella, beginning of the differentiation of the inner integument, in the pollinic sacs the pollen grains are complete developed; 3—4, ovule completely formed, both calottes developed, the macrosporal mother cell is situated towards the nucellus base, the ovarian cavity much restricted; 5—8, divers stages in the development of the embryo-sac, during its development it is consuming the nucella, the both calottes and the internal part of the inner integument too, so that the oosphere apparatus is adhering to the ovary wall; 12, pro-embryo adheres to the ovary wall.

Drawing 1, 3, 8 = 50 X; 2, 4, 5—7, 12 = 240 X. Original.

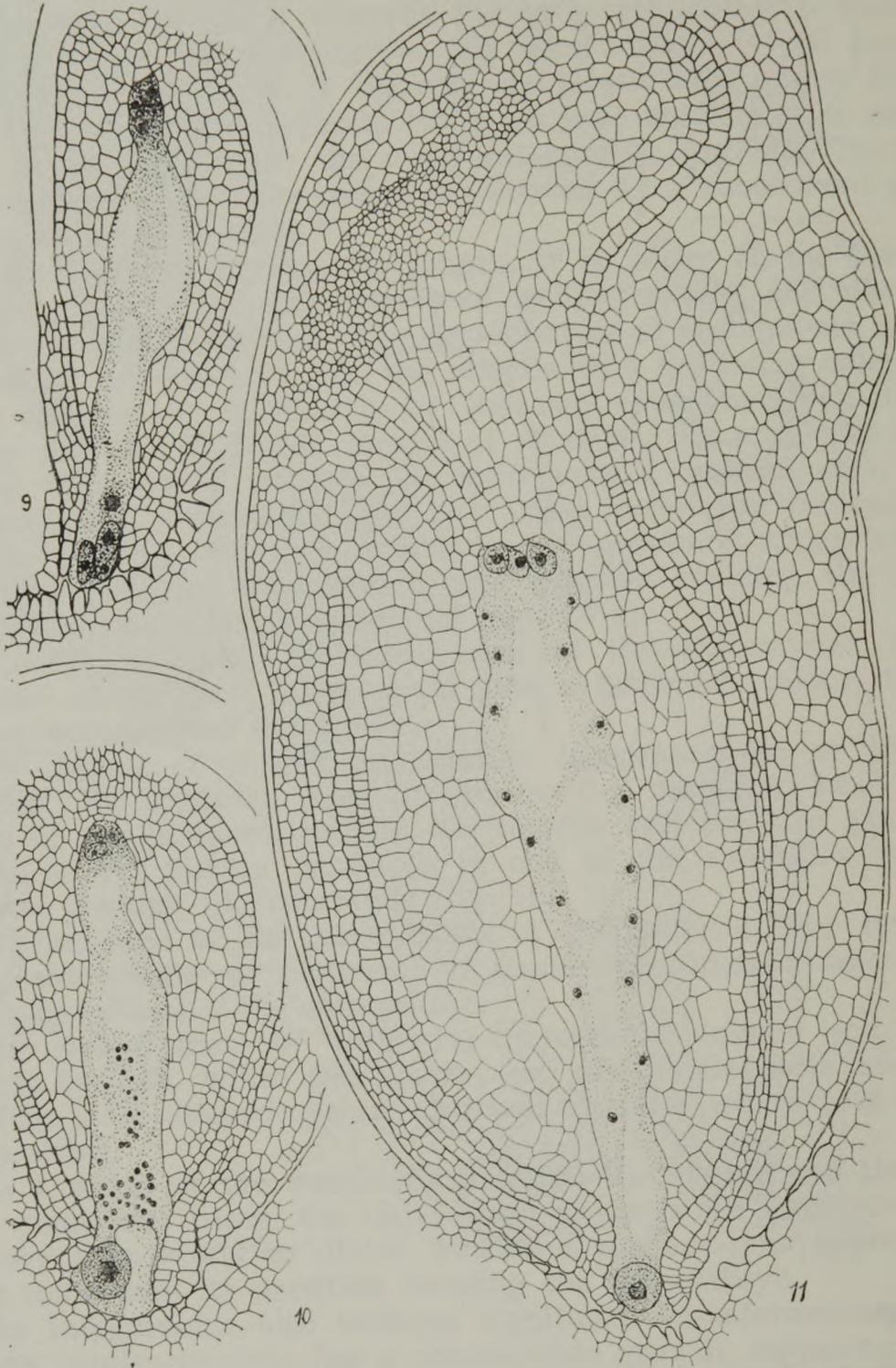


PLATE XVI

Cissus assamica (Laws.) Craib.

Text — fig. 9—11, ovule in longisection. 9, mature embryo-sac; 10, embryo-sac after the process of fecundation, the zygote and the nuclear-type of the secondary endosperm; 11, the ovule increase very much in volume after the fecundation, the antipodes mor or less spherically.

Drawing 240 X. Original.

basis figs 1, 3, 8). The gynoecium is a bicarpellary, bilocular one; in each nest two anatropic-apotropic, ascending, bitegumentate, crassinucellate ovules develop at the base of the carpels. The ovarian cavity is much restricted at the bottom and along the ovule and almost inexistent in the micropyle area, owing to the ovary wall cells, which in this region have the aspect of papillae adhering to the integument (figs. 4, 5, 6, 7).

Out of the prominence taking shape at the base of the carpels there arises the ovule, which starts growing vertically, but later on will bend. Both calottes develop like in *Vitis silvestris*; however, the epidermal one is here built up by only 2—3 cell layers, while the nucellar one by 5—6 cell layers. Owing to the development of the calottes, the macrosporal mother cell becomes finally situated towards the nucellus base. During calotte differentiation the inner integument displays a considerable growth resulting in 3 cell layers at the level of the nucellus, while at its apex in 4—5 cell layers, the latter delimiting a narrow micropyle. The outer integument is developed only in the area opposite to the ovule stalk, is built up by 2—3 cell layers and does not outgrow the apex of the nucellus; therefore, at the stage where the macrosporal cell becomes visible, the ovule is fully developed and will further increase its volume by the lengthening and the enlargement of its cells (figs. 2, 4). The development of the four macrospores and of the embryo-sac out of the basal macrospore happens like in *V. silvestris*, a Polygonum type 8-nucleate embryo-sac being the result. A characteristic feature of this species lies in the fact the embryo-sac during its development grows at the expense of the three apical macrospores, of both the nucellar and the epidermal calotte and leaves the nucellar area after consuming the internal part of the inner integument too, so that eventually the mature embryo-sac is found adhering with its apical part, the oosphere apparatus, to the ovary wall (figs 5, 6, 7).

The embryo-sac is very large, broad for most of its length, but narrower towards the chalaza. The volume increase involved is achieved step by step by consuming the cells of the adjacent tissues. The oosphere apparatus includes the oosphere and two more or less pear-shaped synergids, whose uniform plasma content displays no vacuoles; the oosphere nucleus is basally sited, while that of the synergids, has an apical position. The two polar nuclei unite in the median zone of the embryo-sac, then the resulting secondary nucleus of the embryo-sac makes its way towards the oosphere apparatus (figs 6, 7). The antipodial apparatus is made up by three nuclei in a more or less triangular disposition in the haustorial extension of the embryo-sac (figs 6, 7). With these ovules, when the integuments begin to differentiate, the epidermal calotte acquires a brown colour due to its cells' rich content of reserve substances; this calotte is bound to be consumed during the making up of the embryo-sac. When the embryo-sac is fully developed, the internal epiderm of the inner integument gets brown-coloured, due again to an accumulation of nutrients playing a role in the attraction and feeding of pollinic tubes. At the time the secondary nucleus of the embryo-sac lies in the neighbourhood of the oosphere apparatus, pollinic tubes may be seen in the style and the antipodes increase their

volume, get wrapped in an obvious plasmatic film and assume a pear-shaped aspect before the fecundation process sets in. Antipode differentiation happens at the expense of the cells at the base of the nucellus, in which case the embryo-sac considerably lengthens its haustorial extension towards the chalaza (fig. 9).

The fecundation phenomenon occurs almost simultaneously with the oosphere and with the secondary nucleus by the two gametes brought by the pollinic tube through the ovary wall directly into one of the synergids. Oosphere fecundation by a gamete will bring about the occurrence of a zygote lying close to the ovary wall, among the remains of the inner integument. The zygote does not start immediately dividing. On the contrary, the accessory zygote immediately starts dividing around the zygote, giving rise to numerous nuclei, which within short time will populate the entire embryo-sac cavity, thus giving birth to the nuclear type secondary endosperm (fig. 10).

Beginning with the union of the two polar nuclei and with the formation of the secondary nucleus of the embryo-sac, as well as during the process of double fecundation and of the making up of the nuclear-type secondary endosperm, a considerable growth of the ovule takes place, namely : the basal part of the ovule broadens, the chalaza displaying thereby a basal-ventral position ; the integumental epiderms (in particular the internal epiderm of the outer integument) start differentiating and multiplying ; the nucellus cells greatly expand in volume ; the cells at the embryo-sac base lengthen and get a fan-shaped arrangement directed towards the conductive tissue (fig. 11). The zygote starts dividing when the nuclear-type secondary endosperm starts transforming into cellular endosperm, first at the base of the embryo-sac, then gradually towards its apex ; the pro-embryo arises through repeated division of the egg-cell, adheres to the ovary wall, and the cells of the terminal parts of the integuments will be consumed for its formation (fig. 12).

Though we lacked the material required for an observation of the entire embryo formation, judging by the pro-embryo position in the last stages examined, we might assume in the mature fruit the embryo will neither be surrounded by secondary endosperm nor by the seed tegument arising from integument transformation, but will adhere to the ovary wall, thus maintaining a link through the two basal cells with its food source, the secondary endosperm (fig. 12).

Cissus discolor Bl. (Botanical Garden in Bucharest and in Bogor). Plates XVII and XVIII, figures 1—8.

A native of Java, very popular in greenhouses on account of the particular decorative value of its leaves. These are velvety to the touch and bear silver-bordered spots varying in colour from violet to dark green, while their edges and underside usually are red. From an ornamental standpoint, one might say every leaf is a flower and it has further the advantage of a long life as compared to the ephemeral duration of flowers. As a matter of fact, its small greenish flowers escape observation among the rich colour display of the foliage.

The flowers are tetramerous, the calyx elements are coalescent, the androecium has 4 stamens and the gynoecium is a bicarpellary, coeno-



PLATE XVII

Cissus discolor Bl.

Text — fig. 2—7, flower and ovule in longisection. 2, young ovule with the macrosporal mother cell situated appically; 3—4, the differentiation of the integuments; 5—6, ovule complete developed with a long stalk, the calottes — epidermal and nucelar — have a small extension, in the middle of the nucella there is the macrosporal mother cell; 1, flower in longisection, 4-nucleate stage of the embryo-sac, in the pollinic sac, the pollen grains are complete developed, free.

Drawing 7 = 50 X; 2—6 = 240 X. Original.

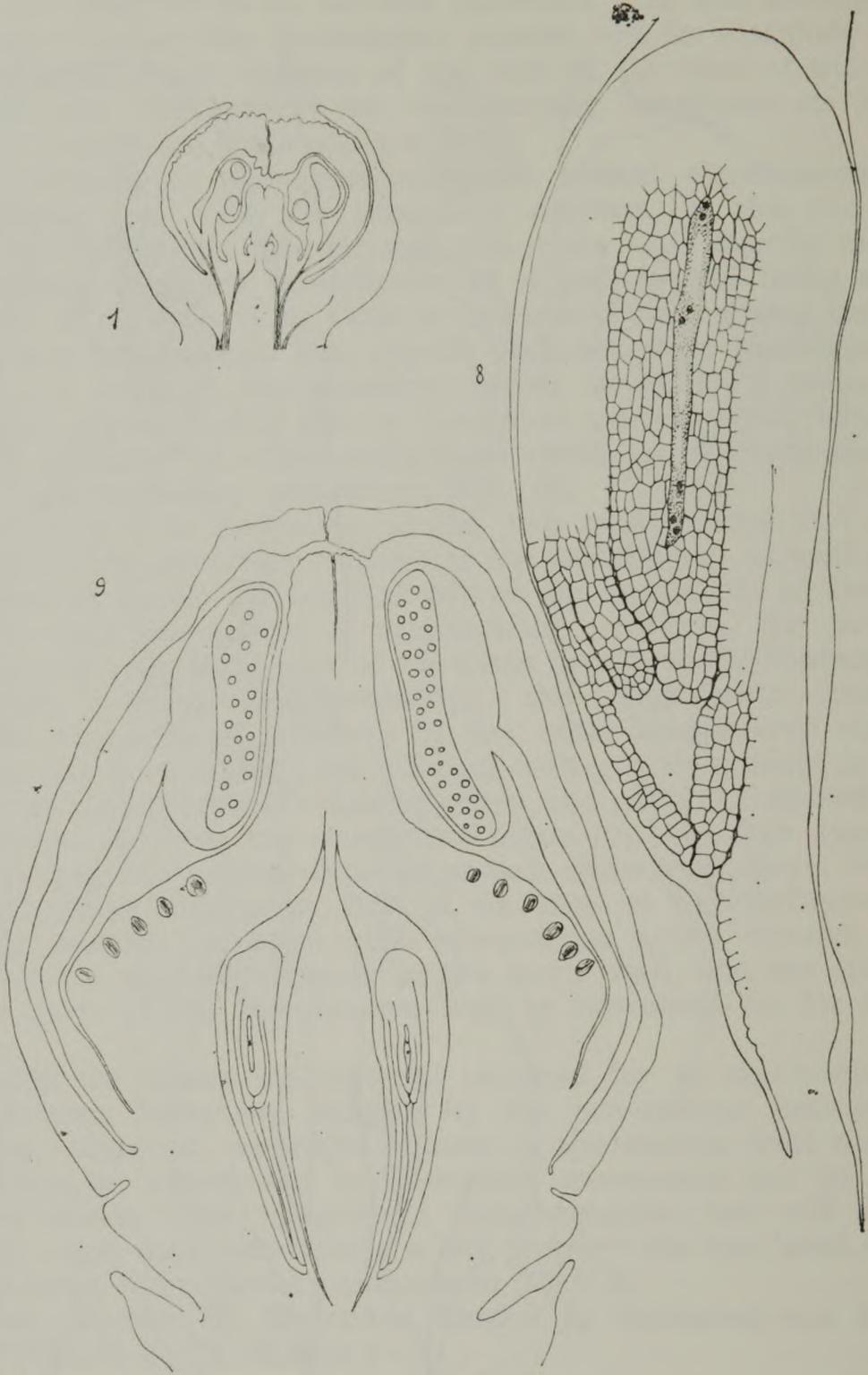


PLATE XVIII

Cissus discolor Bl.

Text — fig. 1, 8, 1, flower in longisection, young ovule; 8, ovule in longisection, 8-nucleate stage of the development of the embryo-sac is the latest stage.

Drawing 1 = 50 X; 8 = 240 X. Original.

Cissus cactiformis Gilg.

Text — fig. 9, flower in longisection, in the pollinic sacs, the pollen grains are complete developed; ovule with long stalk and length integuments, the development of the embryo-sac stops in the mother cell of the embryo-sac stage or 2-nucleate stage.

Drawing 9 = 50 X. Original.

carpic-eusyncarpic, bilocular one. The species is readily multiplied by cuttings, while no seed were ever observed. The sterility of this plant, so far unaccounted for, determined us to investigate its causes.

The research we carried out on its microsporogenesis demonstrated this process runs the normal way, leading to the formation of normal microspores and to viable binucleate pollen grains, which, when mature, are lying free in the cavity of pollinic sacs.

The gynoecium includes a more or less pear-shaped ovary, a very restricted style and a slightly broadened stigma, vaguely bilobate and papillose. In the ovary at the base of the septum, two anatropic-apotropic ascending ovules differentiate in each nest, which is a characteristic feature of all members of the Vitaceae family.

The ovule displays a long stalk, distinct as early as in the first ovule development stages; the ovule primordium born by the stalk grows aslant towards the ovarian cavity, the latter showing in longitudinal section a triangular shape towards the style. In the growth course of this primordium first the inner integument, later the outer one begin to show, then, owing to their unequal development, an incursion of the ovule is determined (figs. 1—3). The inner integument is in this case made up by 3 cell layers, while the outer integument, which is lying opposite to the stalk, is built up by 5 cell layers. Both integuments have a rapid and considerable growth, wrapping up in time the nucellus and displaying in the end a very extensive development in the micropyle area. The outer integument outgrows the inner one; together they will eventually delimit the very long micropyle. Towards the stalk, at the young ovule stage, both integuments are distinct, however, later on, concurrently with the longitudinal growth of the ovule, they get almost entirely coalescent to the stalk (figs 4—6).

Once the integuments are fully developed, calotte differentiation starts in the nucellus and the macrosporal mother cell can be distinctly seen, the latter getting nearer and nearer to the nucellus centre, as calotte differentiation advances (fig. 6). All these phenomena happen in a similar way to that observed in *Vitis silvestris*, however the calottes and, as a matter of fact, the whole nucellus have here rather a small extension, the epidermal calotte being made up of only 2—3 cell layers, while the nucellar one of 3—5 cell layers in a more or less fan-shaped arrangement. When calottes and integuments are fully developed, the relatively small mother cell of the embryo-sac can be detected in the centre of the nucellus (fig. 5), displaying the same width throughout its length; the outset of nuclear division in this mother cell marks the end of the volume increase of the ovule. In the mother cell of the embryo-sac, in parallel to the volume increase of this cell, 4 or even 8 nuclei are formed, however, further organization of the embryo-sac is discontinued. The mother cell is abnormally long and narrow. At this stage a necrosis process sets in involving the stigma and the style, as well as a resorption process of integument cells around the micropyle (figs 7, 8).

Therefore our findings show the sterility in *Cissus discolor* is determined by the embryo-sacs not reaching the mature stage.

Cissus cactiformis Gilg. (Botanical Garden in Bucharest). Plate XVIII, fig. 9.

This is a plant very largely spread in the tropical areas, less so in the subtropical areas, cultivated in greenhouses on account of its decorative aspect. The metamorphosed assimilating, succulent, 4-edged, articulated stalks are mostly devoid of leaves. The latter occur at the articulation points of young stalks and drop away soon. Its flowers are tetra-cyclic tetramerous, bearing a bicarpellary bilocular gynoecium. In each nest two anatropic-apotropic, ascending, bitegumentate, crassinucellate ovules are differentiated.

The ovules develop at the base of the carpels and display the same characteristic features as those in *C. discolor*, namely: very long stalk, much restricted epidermal and nucellar calottes, and micropyle made up both integuments. However, in contrast to *C. discolor*, the ovules of the present species display a much more considerable growth of the outer integument, which surpasses the inner one and runs parallel to the ovule stalk all its length along. Between the two parts of the much extended outer integument and the inner one (the latter having here only a restricted development above the nucellus), there is a more or less wide space along the entire outer integument, which is adhering only in its terminal portion.

Like with *Vitis silvestris*, the first divisions take place in the nucellus centre, where the macrosporal cell is lying, thus situated through the development of the two calottes. Unlike with *V. silvestris*, the evolution of the embryo-sac is stopped at the stage of embryo-sac mother cell, or, at the best, the first division may take place, though the two nuclei stage is very rarely encountered. The volume of this cell is rather considerable: it much extended, uniformly broad all along, surrounded by numerous nucellar cells undergoing disorganization, while the cells at the bottom are at this stage necrotic. Therefore, in this species, ovules and embryo-sacs stop developing much earlier than in *C. discolor*, thereby causing its sterility, though its pollen grains are normally developed and viable (fig. 9).

Cayratia pedata (Lam.) Juss. (= *Vitis pedata* Vahl. ex. Wall.). (Botanical Garden in Bogor). Plates XIX—XXI, figures 1—12.

A native of Africa and New Guinea with tetramerous flowers. The bicarpellary, coenocarpic-eusyncarpic, bilocular gynoecium bears in each ovarian nest two anatropic-apotropic, ascending, bitegumentate, crassinucellate ovules, the ovarian cavity being small around the ovules and almost inexistent in the micropyle area. The ovary is more or less pear-shaped, has a short style and a reduced bilobate stigma. All floral parts are covered with numerous simple, multicellular hairs, while in the nectariferous scale tissue, as well as in the ovary wall, numerous large secretory cells and raphid-containing cells are met.

The ovules start differentiating from the bottom part of the carpels; the prominence containing uniform cells grows initially straight up, then, concurrently with the occurrence of integument primordia, starts bending in the ovarian cavity (figs 1, 2). The inner integument is made of 3 cell layers, while the outer one of 4—5 cell layers; the micropyle is delimited only by the inner integument, since the outer

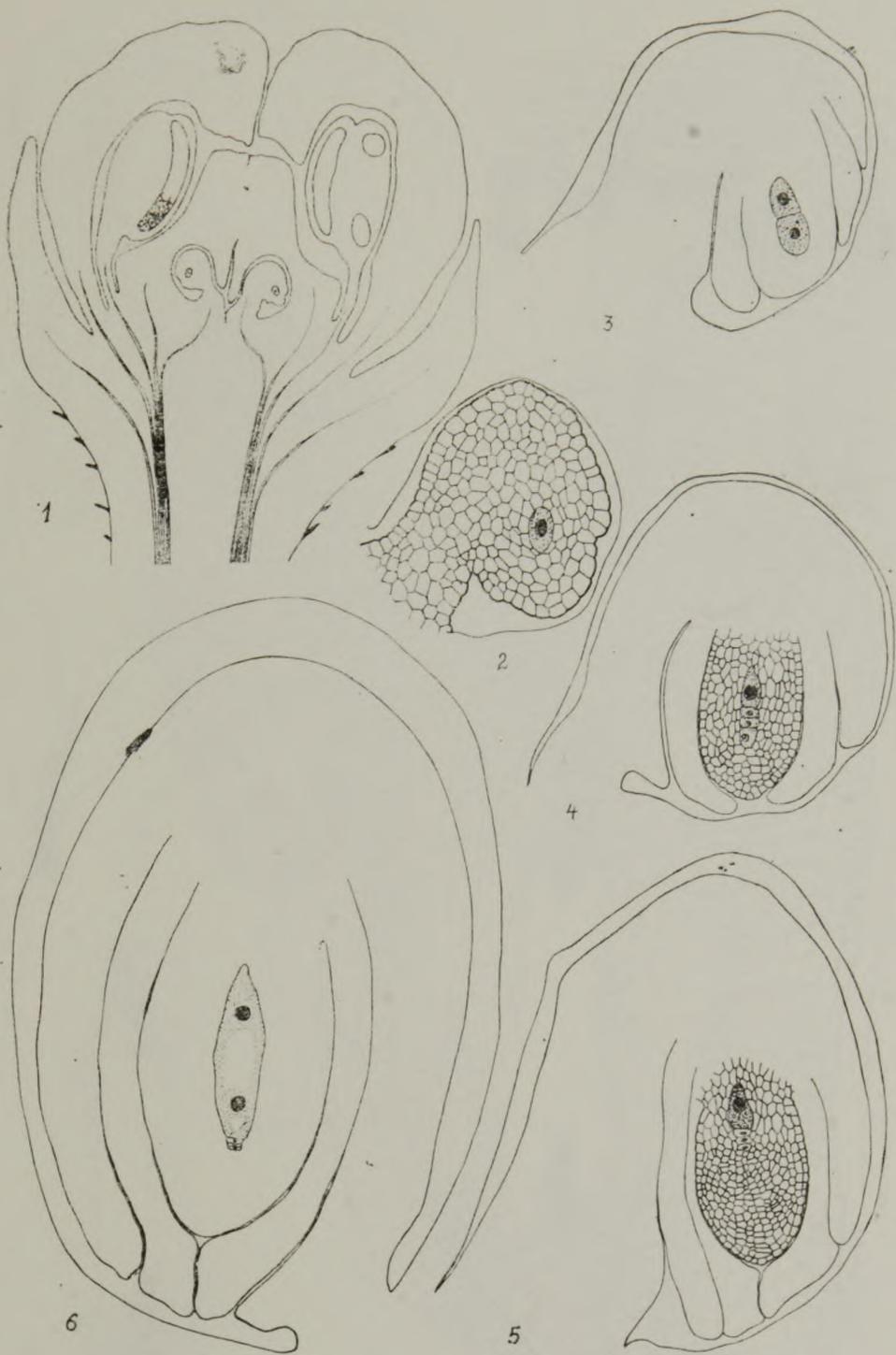


PLATE XIX

Cayratia pedata (Lam.) Juss.

Text — fig. 1—6, flower and ovule in longisection. 1—2, young ovule with the beginning of the differentiation of the integuments and the calottes and the macrosporal mother cell in the middle of the nucella; 3, two macrospores; 4, the linear tetrad of macrospores; 5, the fourth macrospore functioning (mother cell of the embryo-sac), the calottes are differentiated; 6, 2-nucleate stage in the development of the embryo-sac.

Drawing 1 = 50 X; 2—6 = 240 X. Original.

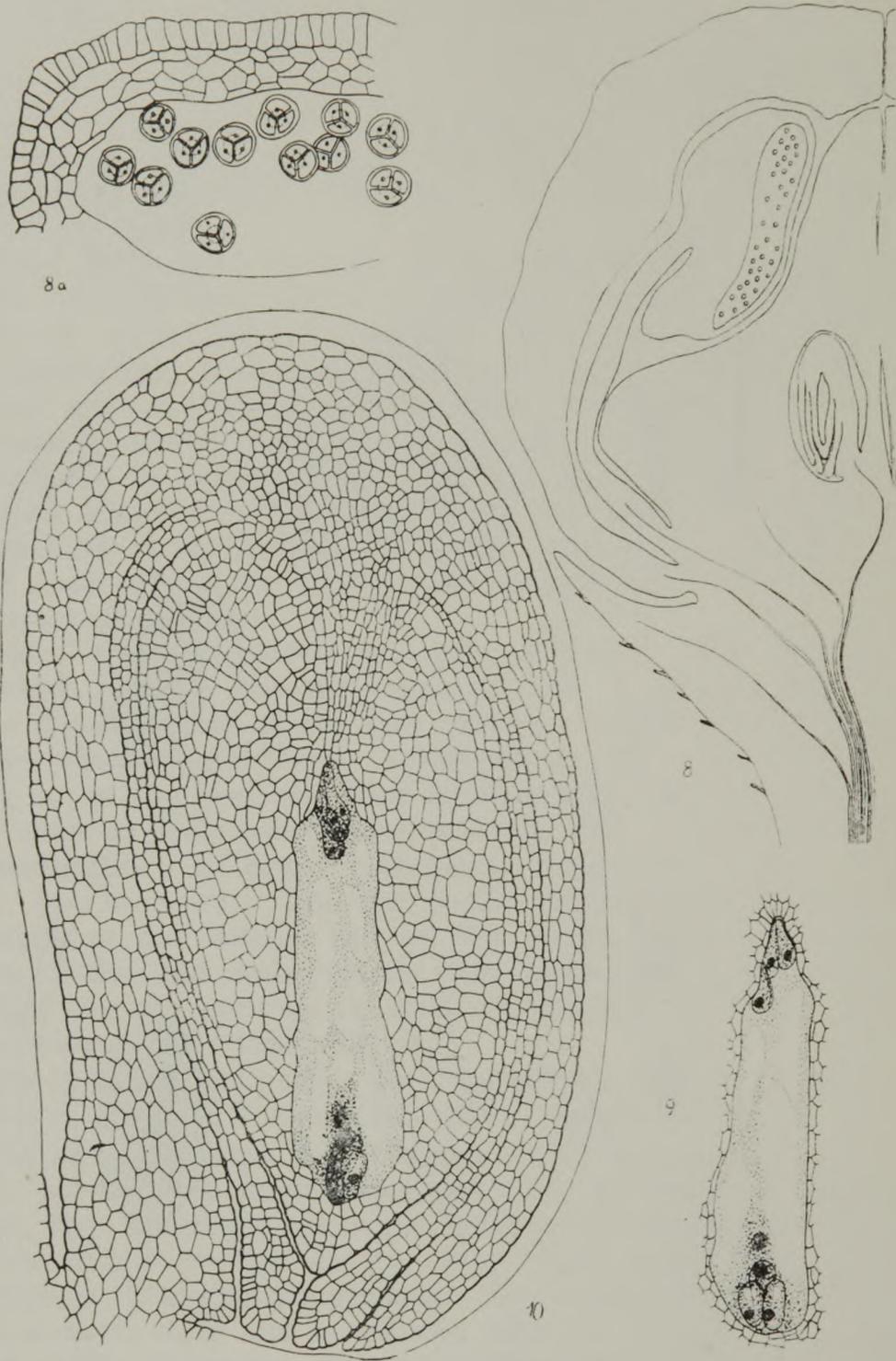


PLATE XX

Cayratia pedata (Lam.) Juss.

Text — fig. 8—10, flower and ovule in longisection. 8—8a, pollen grains in tetrads; 9, embryo-sac complete differentiated, antipodes have more or less pyriform shape; 10, the process of double fecundation, 2-nucleate antipodes.

Drawing 8 = 50 X; 8a, 9, 10 = 240 X. Original.

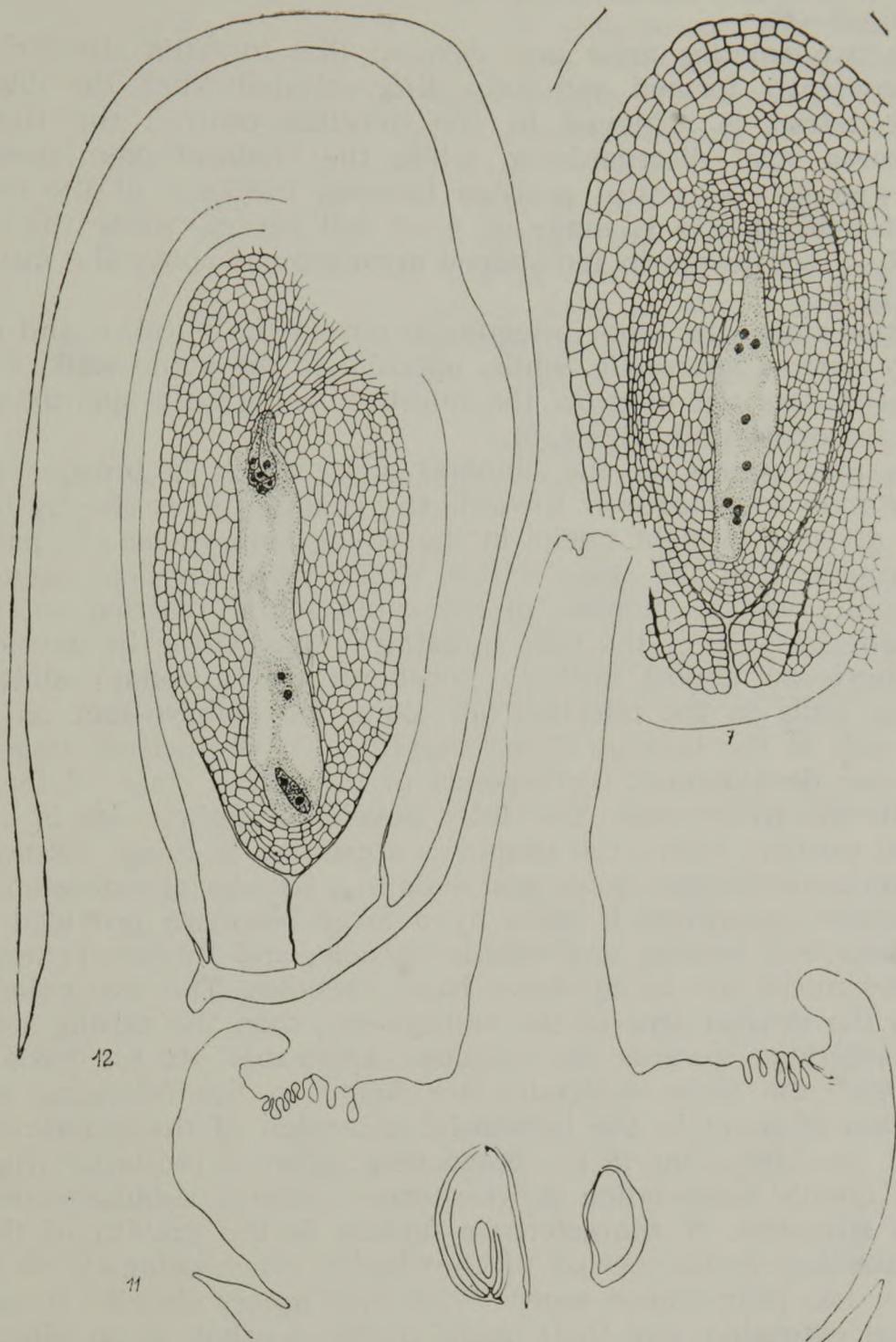


PLATE XXI

Cayratia pedata (Lam.) Juss.

Text — fig. 7, 11, 12, flower and ovule in longisection. 7, ovule complete differentiated, 8-nucleate stage of the embryo-sac ; 11, the aspect of the gynoecium after the process of double fertilization ; 12, the bicellular pro-embryo, the first division of the accesorium zigote, 6 pyriform antipodal cells.

Drawing 7, 12 = 240 X ; 11 = 50 X. Original.

one but by little exceeds the apical area of the nucellus in the mature ovule (figs 2—6).

The two calottes arise and develop like in *Vitis silvestris*: they are concurrently formed and fully differentiated when the linear macrospore tetrad is achieved in the nucellus centre; the first three macrospores start disorganizing, while the chalazal one increases its volume and gets a central position towards the base of the nucellus; the epidermal calotte is made of 4—5 cell layers, while the nucellar one of 7—8 cell layers in fan-shaped arrangement above the macrospore tetrad (fig. 5).

At this stage, the inner integument covers the nucellus and delimits the very narrow micropyle, while, opposite to the ovule stalk, the outer integument does not outgrow the nucellus, being only slightly outlined towards the ovule stalk (figs 4, 6).

The macrospore on the chalazal side, which is broader towards the micropyle and narrower towards the chalaza, gives rise by repeated nuclear division to eight nuclei in the young embryo-sac; in parallel to the enlargement of the space within which these divisions occur, there also occurs a volume increase of the ovule, mainly carried out through the volume increase of the cells building it up. During its development, the embryo-sac almost entirely consumes the nucellar calotte, then numerous cells in the nucellus (all along the embryo-sac), as well as certain cells at the bottom of the nucellus. The 8-nucleate stage of the embryo-sac development corresponds to the tetrad stage of the microspores in the pollen sac. The fully developed embryo-sac is broad in its apical portion where the oosphere apparatus is lying, becomes gradually narrower further down and ends in a haustorial extension (fig. 9). The oosphere apparatus is built by a large oosphere provided with a big nucleus, but lacking any visible vacuole, and by two synergids, in which the nuclei are lying above basal vacuoles. The two polar nuclei unite in the central area of the embryo-sac, then the arising secondary nucleus migrates towards the oosphere apparatus. At the basis of the embryo-sac, the three antipodes are found in the following arrangement: two of them in the haustorial extension of the embryo-sac and the third one higher up, in the broadened region of the latter (fig. 7—9).

The double fecundation process occurs almost simultaneously, like in *Vitis silvestris*. A characteristic feature is the growth of the antipodes: starting in the period of the double fecundation, these assume a more or less pear-shaped aspect, with their apices directed towards the haustorial extension, and their nuclei divide, so that, as an effect, three binucleate antipodes may be observed in the embryo-sac during fecundation (fig. 10). In parallel to the first division of the accessory zygote, which takes place in the centre of the embryo-sac, each nucleus in the three antipodes gets wrapped up in a plasma film of its own, thus resulting in six antipodal cells, while the zygote undergoes its first division, thereby giving birth to a bicellular pro-embryo. Both the fecundation process and the first division of the two xygotes occur almost simultaneously (fig. 12).

In parallel to these alterations inside the embryo-sac, the ovule starts its transformation: the volume increases considerably, the inte-

gumental epiderms begin to differentiate, the ovule basis is much enlarged and the chalaza assumes a ventral-basal position. The cells between the haustorial extension of the embryo-sac and the chalaza have a remarkable longitudinal growth in a fan-shaped disposition. The ovary wall gets appreciably thicker and broader, its borders overlapping the ovary wall tissue made of large raphid-containing cells, while the style and the stigma undergo a necrosis process (fig. 11).

Embryological investigations on this species were carried out by Mulay, Nair and Sastry (1953) — though the investigators do not mention the author's name for the species examined: *Vitis pedata*; we consider, however, this as *Vitis pedata* Vahl. ex Wall., a synonym to *Cayratia pedata* (Lam.) Juss. In our investigations we could confirm in part the data published by the authors mentioned above on the course of the micro- and macrosporogenesis in the species. Among others, they state that the three antipodes lie in a triangular arrangement and very early degenerate, so that during the fecundation process they disorganize and are practically no longer visible.

Now, with the material we examined, the antipodes were clearly visible; not only did they not degenerate during the double fecundation process, but their nuclei divided at the time, so that they became binucleate; then each nucleus with a portion of the plasma got individualized as a plasma film wrapped cell, thus letting eventually six pear-shaped, much lengthened antipodes occur, bearing their narrowed ends in the haustorial extension of the embryo-sac (fig. 12).

Tetrastigma lanceolarium Pl. (Botanical Garden in Bogor). Plates XXII—XXIII, Figures 1—9.

The genus is a native of Indo-Malaysia. It displays two kinds of flowers: male and female. The male flowers have a tetramerous floral envelope, an androecium including 4 stamens, short-filamented in the beginning; in the 4 pollinic sacs of the anthers normal microspores (pollen grains) are formed, at first in a tetrad arrangement, then free in the cavity of the pollen sacs. These flowers also contain a bicarpellary gynoecium with a more or less globular ovary and a much restricted style and stigma. The ovary is a bilocular one, in each ovarian nest there occurring, then starting to develop, the primordia of the two ovules. Their development is continued till the beginning of the integument differentiation and of the ovule incurvation. At this stage, a stagnation of the ovule development sets in and thereby these flowers functionally become male flowers (fig. 6).

With female flowers the differentiation of the anthers ceases at the time when the stamen filament is short, terminally displaying a parenchymatous tissue where no pollen sacs are differentiated, and these stamen rudiments rise only up to the level of the apical portion of the ovary (figs 7, 9).

The gynoecium is here a bicarpellary, coenocarpic-eusyncarpic, bilocular one and in each nest, at the base of the carpels, two anatropic-apotropic, ascending, bitegumentate, crassinucellate ovules are differentiated, the micropyle being delimited only by the inner integument, while the outer one, lying opposite to the ovule stalk, grows as high

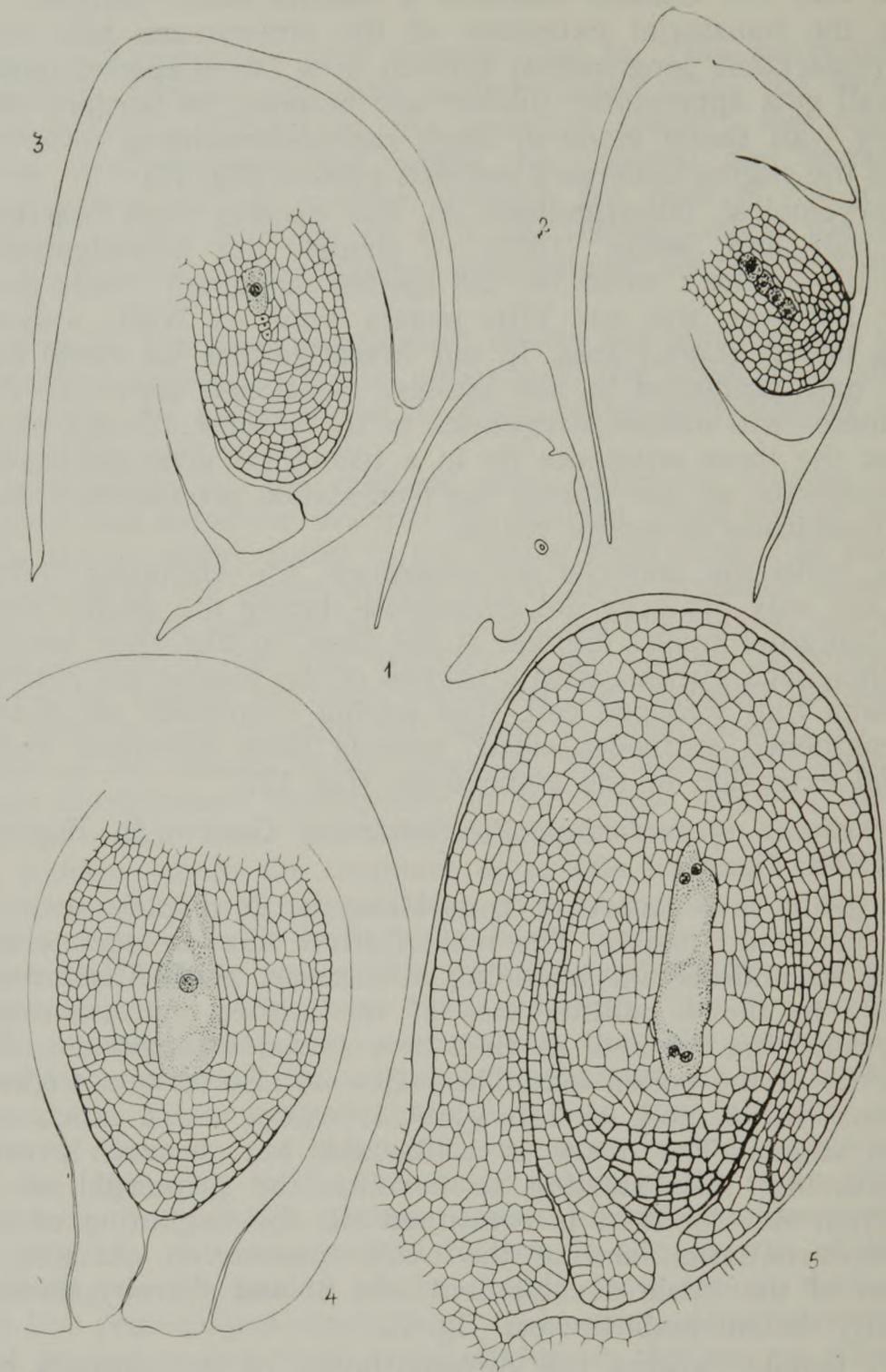


PLATE XXII

Tetrastigma lanceolarium Pl.

Text — fig. 1—5, ovule in longisection. 1, young ovule, with macrosporal mother cell in the middle of the nucella, beginning of the differentiation of the inner integument; 2, young ovule with the linear tetrad of macrospores; 3, the fourth macrospore functioning, differentiation of the calottes and the outer integument; 4, the mother cell of the embryo-sac; 5, 4-nucleate stage of the embryo-sac, the ovule complete formed.

Drawing 1—5 = 240 \times . Original.

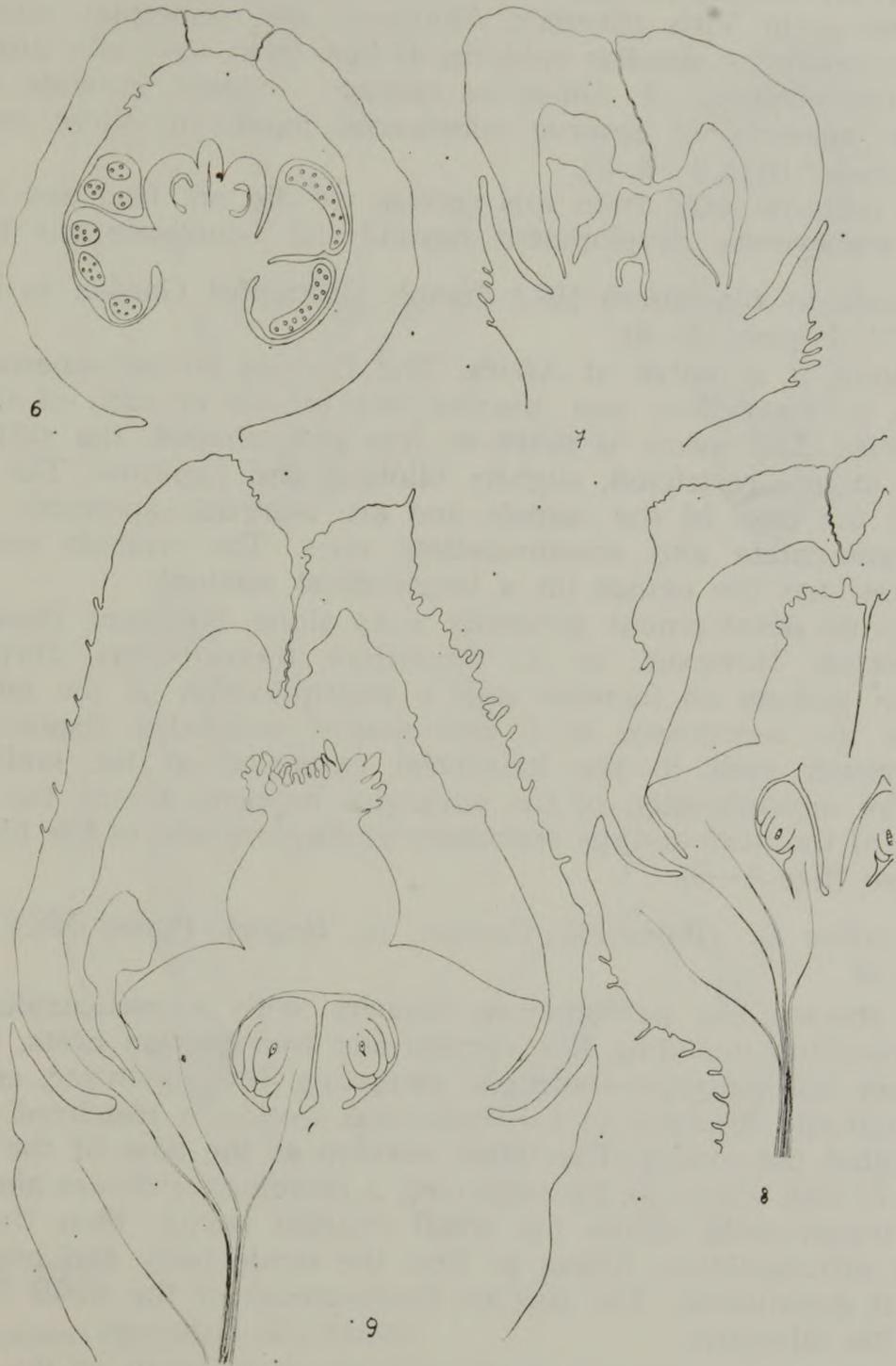


PLATE XXIII

Tetrastigma lanceolarium Pl.

Text — figs 6—9, the female and male flowers in longisection.
Drawing 50 X. Original.

up as the nucellus, but remains only roughly sketched towards the ovule stalk.

The calotte development — the epidermal one, made of 6—7 cell layers, and the nucellar one, made of 4—5 cell layers — follows the same course as in *Vitis silvestris*. However, the epidermal calotte, as well as the remaining nucellar epiderm, is here most obviously displayed, owing to the presence of numerous calcium oxalate crystals and of appreciable amounts of reserve substances imparting these cells an intensive colour (figs 1—4, 8).

In the material used from this species we did not find any further stages of embryo-sac development beyond the 4-nucleate one (fig. 5).

Ampelocissus thyrsoflora (Bl.) Planch. (Botanical Garden in Bogor). Plate XXIV, Figures 1—5).

The plant is a native of Africa. The flowers are tetramerous, the gynoecium a bicarpellary one, bearing two ovules in each of the two ovarian nests. The ovary is more or less pear-shaped, the style very short, the stigma restricted, slightly bilobate and papillose. The ovules develop at the base of the carpels and are anatropic-apatropic, ascending, bitegumentate and crassinucellate ones. The ovarian cavity is restricted around the ovules (in a longitudinal section).

The ovule development generally runs along the same lines as in *Vitis silvestris*. However, in *A. thyrsoflora* there occurs during the fecundation process an increase and a multiplication of the antipodes leading to the occurrence of 6 pear-shaped antipodes disposed with their narrower ends in the haustorial extension of the embryo-sac (fig. 4). The multiplication of the antipodes happens during the partial formation of the nuclear-type secondary endosperm and of the bicellular pro-embryo (figs 4—5).

Leea rubra Bl. (Botanical Garden in Bogor). Plates XXV-XXVI, figures 1—8.

This species has pentamerous flowers with a coencarpic-eusyncarpic gynoecium including five carpels and five ovarian nests, each of which bears one anatropic-apatropic, ascending, bitegumentate, crassinucellate ovule and displays, in a longitudinal section, a restricted ovarian cavity around the ovules. The latter develop at the base of the carpels (figs 1, 3, 8, 8 a), where in the beginning a prominence occurs and grows later on transversely across the small ovarian cavity, then the ovule stalk gets differentiated, lifting at first the ovule body and eventually directing it downwards. The further development of the ovule happens like in *Vitis silvestris*.

With *Leea rubra* the micropyle is built up by both of the integuments, which display a relatively quick growth, particularly the outer one, which outgrows the inner one and participates in the micropyle formation. The inner integument is made up of 3 cell layers, while the outer one has 3—5 cell layers in its portion opposite to the ovule stalk and only 3 cell layers in its portion closer to the ovule stalk. Delimited by both integuments, the micropyle is narrow against the inner integument and broader at the level of the outer one (figs 4, 5).

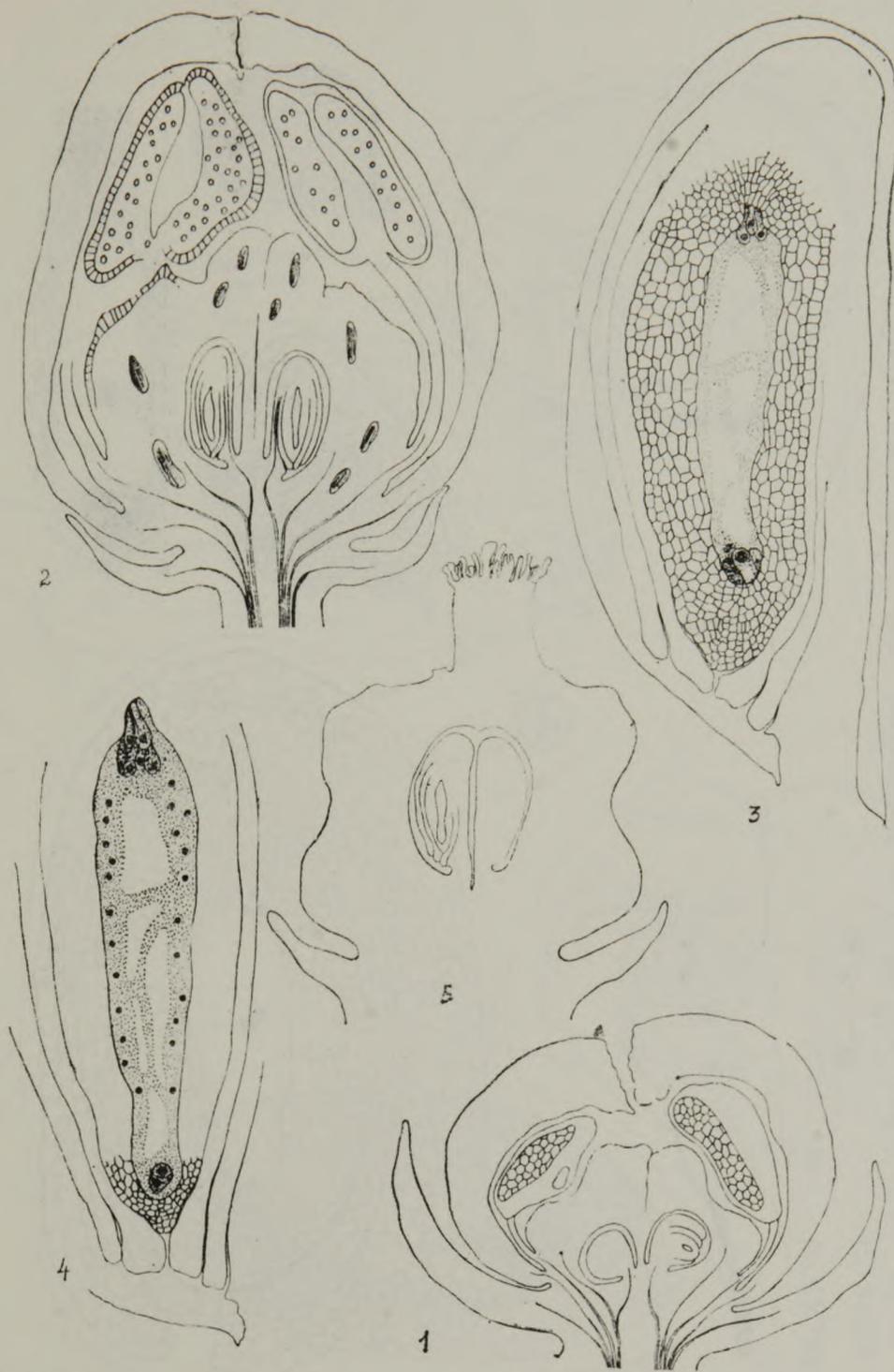


PLATE XXIV

Ampelocissus thyrsoiflora (Bl.) Planch.

Text — fig. 1—5, flower and ovule in longisection. 1, flower in longisection, young ovule with the macrosporal mother cell in the young nucella, in the pollinic sacs the mother cell of the pollen grains; 2—3, flower and ovule in the moment of double fertilization, the beginning of the differentiation of the antipodes; 4, bicells pro-embryo, and the nuclear type of the secondary endosperm.

Drawing 1, 2, 5 = 50 X; 3, 4 = 240 X. Original.

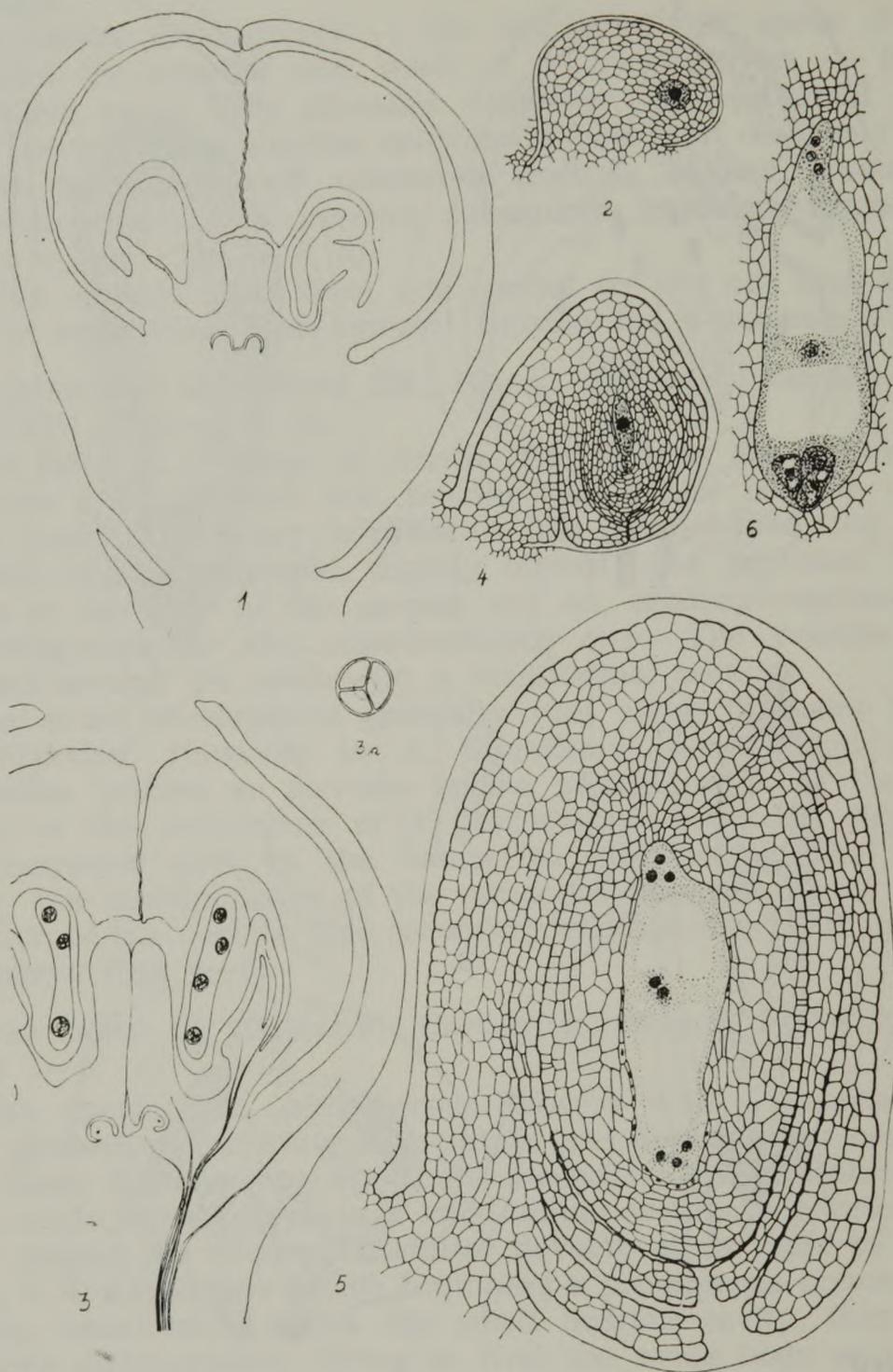


PLATE XXV

Leuca rubra Bl.

Text — fig. 1—6, flower and ovule in longisection 1—3, young ovule with the macrosporal mother cell, in the pollinic sacs there are tetrads of the pollen grains (3—3a); 4, ovule complete formed, the micropyle is built up by both of the integuments, the linear tetrad of macrospores with the fourth macrospore functioning; 5, 8-nucleate stage of the embryo-sac; 6, the mature embryo-sac. Drawing 1, 3 = 50 X; 2, 3a, 4—6 = 240 X. Original.

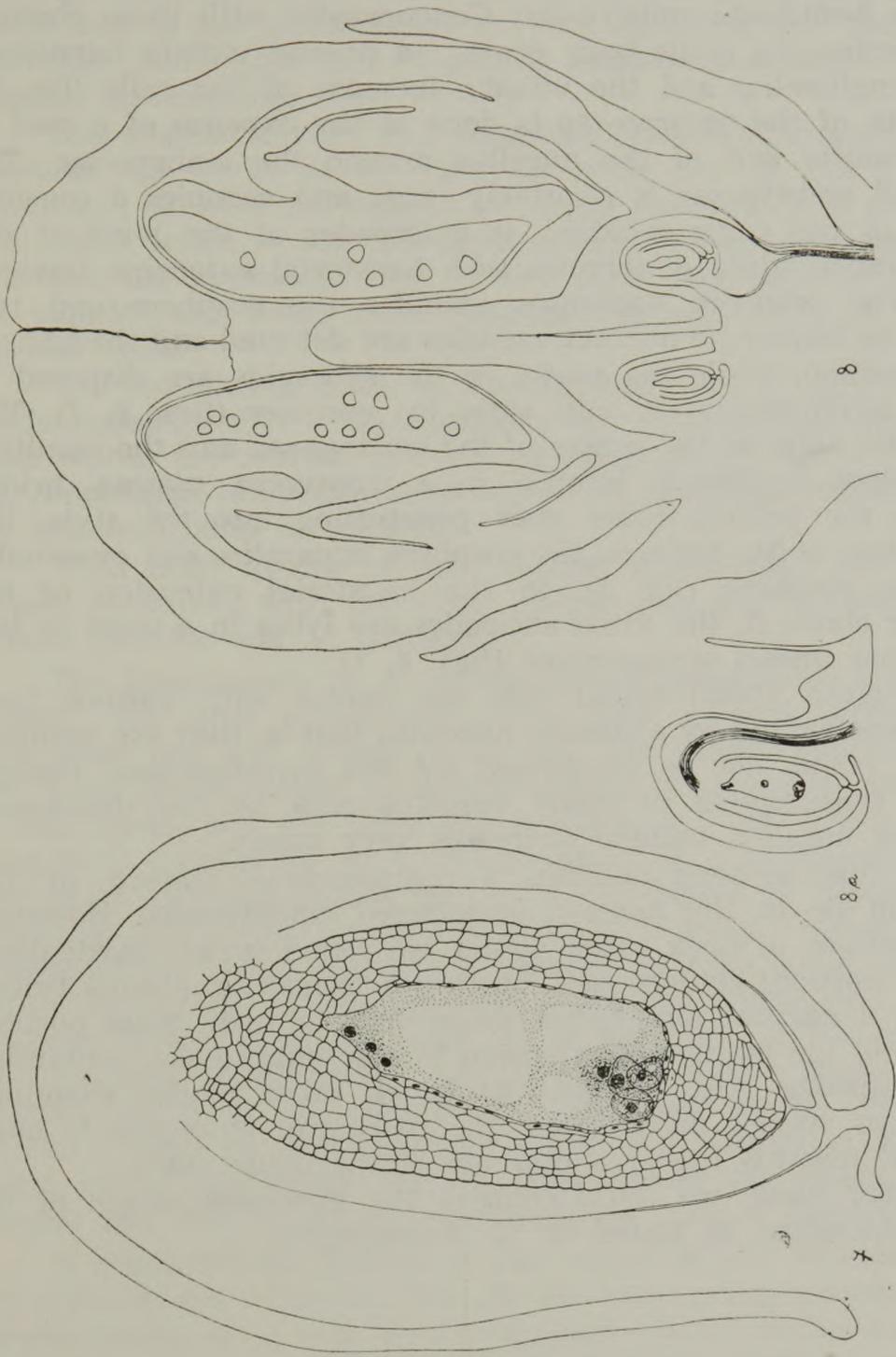


PLATE XXVI

Leuca rubra Bl.

Text — fig. 7—8, flower and ovule in longisection : the mature embryo-sac ; in the pollinic sacs the mature pollen grains.
 Drawing 7 = 240 X ; 8 = 50 X. Original.

When the two integuments have covered the nucellus, therefore the ovule is fully developed, the linear macrospore tetrad gets formed in the middle of the nucellus, the first three macrospores being flattened out, while the deepest lying one displays a relatively big size (fig. 4). The latter macrospore, which shall give birth to the embryo-sac, considerably increases its volume, then its nucleus gives rise, by repeated division, to an 8-nucleate embryo-sac. Concurrently with these changes in the ovule centre, the ovule body grows: a process mainly happening through the lengthening and the volume increase of the cells (fig. 5).

The growth of the embryo-sac is done at the expense of a part of the nucellar calotte and of the nucellus around the embryo-sac. The fully developed embryo-sac is relatively large and occupies a considerable portion of the ovule middle; it is broader at the level of the oosphere apparatus, while it narrows as a haustorial extension towards the chalaza. The oosphere apparatus includes the oosphere and two synergids. In the former no distinct vacuoles are detected and the nucleus has a basal position, while the nuclei in the synergids are disposed in their middle, accompanied on both sides by vacuoles (figs. 6, 7). The two polar nuclei unite in the centre of the embryo-sac and the resulting secondary nucleus is always located in a transverse plasma bridge (fig. 6). When the pollinic tubes start penetrating into the style, the secondary nucleus shifts towards the oosphere apparatus and eventually adheres to the oosphere (fig. 7). In the haustorial extension of the embryo-sac, or above it, the three antipodes are lying in a more or less triangular (if not linear) arrangement (figs. 6, 7).

K. Suessenguth (1935) stated that the ovules with various *Leea* species are characterized by a slender nucellus, that is, they are tenuinucellate, which could not be confirmed in our investigations, though, owing to the consumption of many nucellus cells by the developing embryo-sac the nucellus volume decreases very much.

Again, in that author's opinion, a characteristic feature of the *Leeaceae* would lie in the narrow, lengthened embryo-sacs. However, in our examinations of *Leea rubra* ovules we found broad (particularly in their apical portion) embryo-sacs, bearing a transverse plasma bridge and displaying a narrow, lengthened shape only in their basal portion, where they build the haustorial extension of the embryo sac, containing or not the antipodes. Also, in contrast to all other ovules examined from the various species investigated, the haustorial extension is more or less directed towards the area opposite to the ovule stalk.

On the other hand, we could confirm the gynobasic origin of the ovules in the *Leeaceae*, as stated by K. Suessenguth.

*
* *

The analysis of the female gametophyte development in the 12 taxonomic units investigated: 11 belonging to the *Vitaceae* family, including eight genera, and two cultural varieties of *Vitis vinifera*, and 1 species of the genus *Leea*, the unique genus of the family *Leeaceae*, enabled us to find the Family *Vitaceae* is characterized by bicar-

pellary, coenscarpic-eusyncarpic, bilocular gynoecia bearing in each nidus two anatropic-apotropic, ascending, bitegumentate, crassinucellate ovules.

The ovules develop either at the base of the carpels (obvious gynobasia) as in *Parthenocissus quinquefolia*, *Ampelopsis heterophylla*, *Cayratia pedata*, and in the three *Cissus* species investigated, or out of the basal axil of the partition, as in *Vitis silvestris*, the grapevine cultural varieties "Pinot gris" and "Gordin", *Ampelocissus thyriflora* and *Tetragium lanceolarium*.

The ovarian cavity, in a longitudinal section, is generally restricted towards the style, as in *Vitis silvestris* and in the cultural variety "Gordin", or, alternatively, as in *Cissus assamica*, it is restricted around the ovule, while in the micropyle area it is almost inexistent, owing to the fact the epidermal cells of the carpellary partition have the form of papillae and come quite close to the ovule, that is to the integumental ends.

The prominence made of uniform cells, out of which the ovule will differentiate, grows at first straight up, then assumes an oblique direction within the ovarian cavity; an exception to this is, however, found in *Ampelopsis heterophylla*, where ovule primordia initially have a more or less horizontal orientation.

With most ovules of the species investigated the ovule stalk is short, coalescent with the outer integument, which, however, is long in *Cissus discolor* and *C. cactiformis*.

The integuments in all species investigated have a normal development: the inner integument is the first to start differentiating, then the outer one will follow; as a rule, the inner integument is built up by 3 cell layers, except in *Parthenocissus quinquefolia*, where it displays 4 cell layers, and the outer one is made of 3 cell layers in *C. assamica* and in *C. cactiformis*, of 3—4 cell layers in *Vitis silvestris*, of 4—5 cell layers in *Cayratia pedata*, and of 5 cell layers in *Parthenocissus quinquefolia* and in the grapevine cultural variety "Pinot gris". In most instances, the inner integument exceeds in growth the outer one, covers the nucellus and delimits by itself alone the micropyle; the outer integument grows, as a rule, only up to the level of the apical portion of the nucellus. An exception was found with *Cissus discolor* and *C. cactiformis*, which display abnormally developed ovules, while the integuments therein (in particular the outer one) display a stronger development in the micropylar portion, reach about two thirds of the nucellus length and delimit a long and relatively narrow micropyle.

A characteristic feature of the ovules of the Vitaceae family members lies in the following fact. In the very young nucellus (in the ovule primordium), as a rule at the time the inner integument starts differentiating, the archesporal cell gets individualized under the epiderm. By division, this will give birth to a primary parietal (covering) cell and, towards the inside, to the macrosporal mother cell. By repeated periclinal and anticlinal divisions, the primary parietal cell gives then rise to the nucellar calotte, which includes a number of cells varying with the species concerned; thus, in the grapevine cultural variety

"Pinot gris" it has 10—15 cells, in *Parthenocissus quinquefolia* 10—12, in *Cayratia pedata* 7—8, in *Vitis silvestris* 5—7, in *Cissus assamica* 5—6, while in *C. discolor* and in *C. cactiformis* only 2—3 cells.

Concurrently with the formation of the nucellar calotte, apically out of the epidermal cells of the nucellus there arises by periclinal divisions the epidermal calotte, which is more developed in the grapevine cultural varieties "Pinot gris" and "Gordin" and in *Tetrastigma lanceolarium* (6—7 cell layers), or less developed, as in *Vitis silvestris* (3—5 cell layers); further, it displays 4—5 cell layers in *Ampelopsis heterophylla* and in *Cayratia pedata*, and 2—3 cell layers in *Cissus discolor* and in *C. cactiformis*.

The presence of the epidermal and nucellar calottes was reported by Baranov (1946), by Mulay, Nair and Sastry (1953) and by Nair and Bajaj (1966).

Through the development of the epidermal and nucellar calottes, whose cells display a more or less fan-shaped arrangement, the macrosporal cell gets a situation deep in the nucellus, more often in its middle, but sometimes at its very bottom.

In proportion to the growth of the ovule body, the macrosporal cell greatly increases in comparison with the cells of the nucellus, is rich in plasma and contains a large nucleus. By division, this cell gives birth to a linear tetrad of macrospores, out of which the three next to the micropyle degenerate, while the basal one considerably increases its volume, assumes a lengthened shape, broader towards the micropyle and much narrower towards the chalaza, and finally, by repeated division of its nucleus, gives rise in its turn to the eight nuclei, which will constitute the embryo-sac, which is characteristic for the Vitaceae family. The embryo-sac is, therefore, an 8-nucleate Polygonum-type monosporic one.

The first division of the nucleus in the fourth macrospore, which thus became the mother cell of the embryo-sac, results in the occurrence of two nuclei, which make their way each towards one end of the cell, separated by 1—2 big central vacuoles. Presently they divide again, first two, then four nuclei arising at each cell end. Among these, three will remain at the micropylar end, constituting the oosphere apparatus, and three will remain at the chalazal end, building there the antipodal apparatus. One nucleus from each end will migrate towards the cell centre: they are the polar nuclei. Concurrently with the organization of the embryo-sac a considerable volume increase of the ovule takes place; this is mainly achieved by a volume growth of the cells, while the volume increase of the embryo-sac is achieved both by its lengthening and by consumption of part of the nucellar cells surrounding it.

The oosphere apparatus involves a large oosphere in a central position, containing a big nucleus at the base end, as a rule, an apical vacuole, and two synergids on each side of the oosphere, containing each a basal vacuole and an apically situated nucleus.

A uniform content due to the absence of vacuoles was observed both in the oosphere and in the synergids with *Ampelopsis heterophylla* and *Cissus assamica*.

The two polar nuclei reach the centre of the embryo-sac and unite there, giving rise to the secondary nucleus of the embryo-sac, which makes its way towards the oosphere apparatus and settles immediately close to the oosphere: this happens in the grapevine cultural variety "Pinot gris", in *Parthenocissus quinquefolia*, in *Cayratia pedata*, and in *Cissus assamica*. Alternatively, the two polar nuclei, lying in a dense plasma pouch, move closely together towards the oosphere apparatus, in whose vicinity they unite giving birth to the secondary nucleus of the embryo-sac, which then shifts still closer to the oosphere: this happens in *Vitis silvestris*, in *Ampelopsis heterophylla*, in *Tetrastigma lanceolarium*, in *Ampelocissus thyrsoflora* and in the grapevine cultural variety "Gordin".

The fully developed embryo-sac has a central position in the nucellus, being protected towards the micropyle by several cell layers, namely by the epidermal calotte and by a rest of the nucellar calotte. In *Cissus assamica* the embryo-sac entirely consumes, during its formation, both the nucellar and the epidermal calottes, as well as part of the inner integument around the micropyle, thus reaching the ovary wall, which in this instance is adhering to the ovule. The oosphere apparatus in the same species is situated among the remains of the inner integument, adhering to the ovary wall, hence it fully leaves the nucellus.

The embryo-sac generally has a lengthened shape, broader towards the apex, somewhat narrower in the middle, then slightly broader again, and eventually ending towards the chalaza under the form of a haustorial extension. An exception was found with the embryo-sacs in the grapevine cultural varieties: „Pinot gris“, which were uniformly broad all along, excepting the haustorial extension, and „Gordin“, which were long and narrow, while in the chalazal area very narrow.

The pollinic tube brings the two gametes into the embryo-sac by penetrating the micropyle: porogamy was found in most taxonomic units investigated. However, with *Ampelopsis heterophylla*, from the ovary wall the pollinic tube reaches the nucellus not through the micropyle, but across the top of the two integuments, while in *Cissus assamica*, where the embryo-sac emerges from the nucellus with its apical portion (the oosphere apparatus) and lies between the ends of the integuments and the ovary wall, the pollinic tube penetrates one of the synergids directly out of the ovary wall.

Having reached in one of the synergids together with a large plasma amount, the two gametes fecundate the oosphere and the secondary nucleus of the embryo-sac (which lies close to the oosphere) almost at the same time: this happens in a similar way in all taxonomic units investigated.

In most of these taxonomic units the resulting zygote does not immediately divide — with the sole exception found in *Cayratia pedata*, where the zygote divides concurrently with the accessory zygote.

Directly upon fecundation, the accessory zygote moves towards the base of the embryo-sac, where it starts dividing, thus giving rise to a nuclear-type secondary endosperm, at first in the basal portion of the embryo-sac, later on in its apical portion. As long as the stage of

a well developed nuclear endosperm persists, the zygote goes on dividing, eventually producing a bicellular pro-embryo.

Baranov (1946), Mulay, Nair and Sastry (1953), Nair and Bajaj (1966) state in their embryological studies carried out in various Vitaceae species, that the antipodes get disorganized even before the fecundation process.

However, we found in all members of the Vitaceae family we investigated that the antipodes not only persist throughout the fecundation process, but increase their volume before or immediately after completion of the double fecundation process; further, that they are well delimited by a plasma film, may even contain vacuoles (thus in the grapevine cultural variety „Pinot gris“); they may display a spherical shape, as in grapevine variety „Gordin“ and in *Parthenocissus quinquefolia* (in the latter their number being reduced to 2 through the disorganization of the third one) or, alternatively, be pear-shaped: two of them in the cultural variety „Pinot gris“ or 3, as in *Vitis silvestris*, *Ampelopsis heterophylla*, *Cissus assamica*, and *Tetrastigma lanceolarium*. A particular instance is offered by the antipodes of *Cayratia pedata* and of *Ampelocissus thyrsoflora*, which multiply during the fecundation process, giving rise to six lengthened pear-shaped antipodes.

The existence of the antipodes, their very increase and multiplication, tend to support the conception of a role they might play in the taking up of nutrients in the chalazal area, of the substances required for the development of the embryo-sac and for the differentiation of the secondary endosperm and of the pro-embryo (embryo). This conception is further supported by the embryo-sac basis being shaped as a haustorial extension, as well as by the aspect of the tissue at the base of the embryo-sac: a tissue made of cells lengthened in a fan-shaped arrangement towards the chalaza.

The changes occurring in the ovule and in the ovary subsequent to the fecundation (particularly those in the former), as found in *Vitis silvestris*, in *Cayratia pedata* and in the grapevine variety „Pinot gris“, confirm the data published by Baranov (1946).

As to the time required for the pollinic tube to grow and reach the embryo-sac with its two spermatid nuclei in order to let the fecundation process take place, we could find no data in the literature, except Baranov's statement (1946) that „the effects of fecundation may be observed in the grapevine as early as the next day following pollenization, materialized in the visible increase of the ovary“.

It is rather difficult to establish the fecundation time in the Vitaceae, since the flowers in an inflorescence don't reach maturity at the same time. We carried out artificial pollenization tests with the grapevine varieties „Pinot gris“ and „Gordin“, and with *Vitis silvestris*. By analyzing the artificially pollenized gynoecia sampled and fixed every 15 minutes after pollenization, we could find that the completion of the fecundation phenomenon takes 60 minutes with *Vitis silvestris* and 15 minutes with both grapevine cultural varieties mentioned.

Out of the four ovules present in the ovary, almost always only one (seldom two) reaches maturity and gets fecundated, yielding 1—2 seeds, respectively.

The sterility phenomenon is quite frequently met with Vitaceae species. This may be due either to the formation — through anomalies in the microsporogenesis process — of sterile pollen grains, or, alternatively, to the development of ovules, whose embryo-sac does not reach its fully developed stage, as occurring in *Cissus discolor* and in *C. cactiformis* where the embryo-sac do not develop beyond the 2—4 (rarely 8) nuclei stage, so that no typical embryo-sac can be formed.

Discussion and general conclusion

The Rhamnales Order, such as delimited in the strictest sense by Engler (1892, 1897), that is made up by the families Rhamnaceae and Vitaceae (including genus *Leea*), was diversely situated in the various phylogenetic worked out so far.

Morpho-palynological and embryological investigations carried out in this study have put forward new morphological characters in support of the taxonomists who placed the Rhamnales Order as an ultimate point of evolution, its latest development paralleling that of the Celastrales Order.

The pollen grains from all the investigated members of the families Rhamnaceae, Vitaceae, and Leeaceae have many properties in common, which demonstrates the unity of this order. These properties are apparent in the shape of the pollen grains; in the presence of three colpi delimited all along by a fold formed by a turning of the exine, which sometimes builds up a ridge around the pores; and, finally, in the strong relationship displayed by the epistucture of the sporoderm and by its structure as seen in optical section.

The tri-colporate type of the pollen grains is only encountered in the more evolved Angiosperm groups, among which the Rhamnales Order is also to be considered.

The morphology of the pollen grains in the three investigated families of the Rhamnales Order shows a close relationship to the Celastrales Order (in particular with the Celastraceae and Hippocrateaceae) and supports the opinion of some taxonomists such as Bush (1940) and Emberger (1960), who consider these orders to have derived from a common ancestor and evolved in parallel.

The presence of trinucleate (tricellular) pollen grains in *Vitis longifolia* and in the productive hybrid "Isabelle" is a further indication of the advanced character of this group of plants, since this pollen type is considered as the most differentiated type of the male gametophyte in the Angiosperms. Schürhoff (1926), Gerassimova-Navashina (1961), Poddubnaya-Arnoldi (1964), Maheswari (1950—1963), and Rudenko (1965) consider the division of the generative nucleus inside the pollen grain before the latter's parting from the sporophyte to be the last stage in the evolution of the male gametophyte in the Angiosperms and they also point out the tendency to build trinucleate (tricellular) male gametophytes was always observed only in the more differentiated plants, while the overwhelming majority of the Angiosperms display binucleate (bicellular) pollen grains. The restricted and disseminated

distribution of the trinucleate (tricellular) pollen grain character corroborates the opinion according to which the presence of binucleate (bicellular) pollen grains is a sign of primitivity.

On the other hand, if the peculiar features of the male gametophyte development in some members of the family Vitaceae are considered, certain superiority characters can be found. Thus, taking into account Gerassimova-Navashina and Meier's assertion (1953) that the process of the male gametophyte development consists in its gradual reduction down to the complete disappearance of the vegetative part, then the outstripping of the vegetative cell by the generative one during pollen germination in the *Ampelopsis* species investigated or its resorption along the pollinic tube indicate morphological properties specific to the plant groups of higher organization.

An analysis of the morphological characters of the gynoecium, of the ovule and of the female gametophyte in some members of the families Vitaceae and Leeaceae also discloses superiority features in this plant group, namely the restricted number of ovules, the presence of conductive tissue mostly uniquely in the ovule stalk (in some instances it ramifies in the chalaza); the Polygonum-type monosporous embryo-sac; the presence of antipodes sited in haustorial extension of the embryo-sac.

In papers concerning the female gametophyte development in this plant group — Baranov, 1946; Mulay, Nair and Sastry, 1953; Nair and Bajaj, 1966; Dvornic, 1961 — it is stated the antipodes are short-lived, being resorbed before or during the fecundation process.

However, we found in our investigations that antipodes continue to exist, increase their volume, get wrapped in an evident plasmatic film, become spherical or pyriform in shape, persist during the fecundation process and afterwards, in some cases they even can multiply. They are always located in the haustorial extension of the embryo-sac, while the tissue at the basis of the embryo-sac is built up by cells prolonging in fan-shaped arrangement towards the chalaza.

The differentiation of the antipodes and of the basal tissue of the embryo-sac during the fecundation process supports the opinion of authors who ascribe to them an important physiological role in the transportation of nutrients to the embryo-sac, where the differentiation of the secondary endosperm and of the embryo occurs, and this is considered as a superiority feature evincing a higher specialization and a better adaptation of the nutritive function (Kreda, 1902; Alexandrov and Alexandrova, 1946, 1952; Maheshwari, 1950; Kostriukova and Guretskaya, 1956; Tsigler, 1958; Poddubnaya-Arnoldi, 1959). The last two authors carried out histochemical investigations on the tissue at the basis of the embryo-sac and demonstrated in the area a high physiological activity, the feeding of the embryo-sac with physiologically active substances such as enzymes.

The presence prior to the fecundation process, in the epidermis of the nucellus or in the interior one of the inner or of the outer integument of substances imparting to them strong colours, reported as early as 1923 by Netolitzky, was considered as an inferiority element, the dyeing substances being thought to be excretion products useless

to the plant. Histochemical investigations performed by Tsigler and Poddubnaya-Arnoldi (1959) and by Poddubnaya-Arnoldi and Petrovskaya-Bazanova (1959) demonstrated these cells were rich in albumins, nucleoproteins, amino acids and glucides — similarly to the tapetum cells in the anther — and they were ascribed an active physiological role in the attraction and feeding of pollinic tubes. This is a property specific to the highly evolved groups like the Compositae and the Orchidaceae. The presence of such substances in the epidermis of the nucellus or in that of the inner and of the outer integument in most ovules during the formation and the maturation of the embryo-sac in the investigated members of the families Vitaceae and Leeaceae gives strength to the opinion this plant group is of a higher organization.

Although the members of this plant group also display certain inferiority traits such as the crassinucellate and bitegumentate ovules and the existence of epidermal and nucellar calottes, the fact that advanced morphological characters are predominant (the pollen grain type and various details in the development of the male and female gametophytes) enable us to conceive this order as possessing a superior organization, which justifies its terminal position in an evolution branch as suggested by some authors.

Inside the Rhamnales Order, even as initially outlined by Engler (1892), the family Rhamnaceae is clearly circumscribed. It must be remarked that the morphological characters regarding both the vegetative and the reproductive spheres, which impart this family its natural unity, are running in parallel to its morpho-palynological features. On the morphological structure of pollen grains in members of this family we only have Erdtman's statement, 1952, asserting that their properties are identical with those of the pollen grains in members of the Vitaceae family, though the exine stratification is here more or less indistinct. In the morphological study we performed in eight species belonging to three genera of this family, we were able to ascertain the structural unity of the family in this respect again. This uniformity is expressed in the restricted size of the pollen grains, in their pale yellow hue, in the thinness of the sporoderm (about one micron), and in the exine bearing more or less long pili ending in spherical capita.

Taking into account the morpho-palynological and the embryological features of the investigated *Leea* species and various Vitaceae genera, we adhere to the taxonomists who grant genus *Leea* the standing of an independent family within the Rhamnales Order, because, along with many similarities to certain species belonging to Vitaceae genera, genus *Leea* displays sufficient distinct characters pleading for its segregation in order to build up an independent family. Among the distinct morpho-palynological characters of *Leea* species we would mention: the (medium to) big size of the pollen grains ($P = 55-61 \mu$) — in contrast with Vitaceae species, which are characterized by small or medium size pollen grains (medium size at its upper limit was found with only two species among the 112 taxonomic units investigated) —; the brown colour of the pollen grains (a colour found among the Vitaceae only with *Tetrastigma* species); the usually shorter colpi, broader at the level of the pores and always sharpened at the ends; the $2-3,5 \mu$

thickness of the sporoderm, while with the Vitaceae the sporoderm is mostly finer : 1—1.5 μ , rarely exceeding 2 μ ; the nexine is two-layered in all *Leea* species investigated — a property never encountered in Vitaceae species ; the lids over the pores in most pollen grains of the Leeaceae, a feature only incidentally met in the Vitaceae.

To these distinct morpho-palynological features we should add several others concerning the reproductive sphere.

The members of the Vitaceae family are characterized by a coenocarpic bicarpellary gynoecium with two ovarian nidi, each containing two ovules, while the coenocarpic gynoecium of the Leeaceae is built up by 3—5 carpels delimiting 3—5 ovarian nidi each containing one ovule.

As to the type of the ovule and of the embryo-sac, there are no differences between the members of the two families. All of them have anatropic-apotropic, ascending, crassinucellate and bitegumentate ovules displaying a monosporous Polygonum-type embryo-sac. However, while in all the ovules of the Vitaceae species investigated the micropyle is very narrow, delimited solely by the inner integument (the outer one does not grow up to the inner one's length) and the ovule body is lengthened, in the *Leea* species the broad micropyle is delimited by both integuments and the ovule body is more or less globular.

It should also be noted that in the eight-nucleate Polygonum-type embryo-sac common to both families, with *Leea* species a cross plas-matic bridge is found, situated more or less in its centre, where the secondary nucleus is lying, while with Vitaceae species the embryo-sac displays several vacuoles, but never such a transversal plasma bridge.

The existence of numerous morphological characters in common, both morpho-palynological and embryological, in the Vitaceae and the Leeaceae demonstrates their close relationship ; on the other hand, the morphological differences with respect to the morphological structure of the pollen grains, as well as to the ovule development and to the female gametophyte structure, all mentioned above, along with various other features in the vegetative sphere justify, in agreement with Gagnepain, Wettstein, Suessenguth and Emberger, the separation of genus *Leea* from the Vitaceae and its raising to the rank of an independent family within the Rhamnales Order. This opinion is also supported by M. Reille (1967) in his palynological study on 52 species belonging to the Vitaceae and the Leeaceae.

The relative position of some species belonging to various genera inside the Vitaceae family was much discussed. Indeed, it would be interesting to ascertain whether the respective genera, *Vitis*, *Cissus*, *Ampelopsis*, constitute or not natural units and, in particular, whether the whole caryological, morpho-palynological and embryological data accumulated justify or not the delimitation of the genera *Parthenocissus*, *Cayratia* and *Ampelocissus*.

Caryological investigations we undertook enable us to state that genus *Ampelopsis* has a unitary character, all its species we studied, as well as others commented in the literature, displaying the same chromosome number, $2n = 40$.

Caryologically unitary is also genus *Vitis*, since in all its species or varieties or cultural forms we investigated the chromosome number was $2n = 38$. The literature data indicate for most species of the genus $2n = 38$, however, in older papers such as Dorsey (1914), Sax (1929), Branas (1932), $2n = 40$ is reported for *Vitis bicolor*, *V. labrusca*, *V. riparia*, though in the same species more recent reports give again $2n = 38$. In *Vitis vinifera* most of the investigators found $2n = 38$, although various other papers, either recent or of older date, report different chromosome numbers, namely $2n = 40$, 57 or 76. In our opinion, in consideration of the small size of the respective chromosomes and of difficulties met in determining their exact number, the first figure mentioned above ($2n = 40$) might be an erroneous one, while the numbers 57 and 76, thought to be characteristic for the species concerned, might only reflect the fact hybrid or polyploid forms were investigated. On the whole, we consider genus *Vitis* caryologically unitary. The 8 cultural varieties we examined displayed all of them the same chromosome number, $2n = 38$, though to this purpose we selected varieties bred in different geographical and climatic conditions.

The genus *Parthenocissus*, both the species we studied and those reported in this respect in the literature, has $2n = 40$ chromosomes, which shows the plants grouped therein are closely related, thus the genus is a unitary one, differing from genus *Vitis*, while closer to the genus *Ampelopsis*.

Among the Vitaceae, genus *Cissus* displays the widest variation with respect to the chromosome number, namely, for the various species investigated $2n$ was reportedly found to be 22, 24, 26, 28, 32, 45, or 48 chromosomes. This diversity in the chromosome number indicates the heterogeneity of the plants grouped under this heading. However, Shetty (1959) considered after a comparative analysis of the caryotypes of 7 species of the genus, that the chromosome morphology of these species pleads for their being kept together in spite of the differing chromosome number. Among the species we investigated, *C. glauca* and *C. cactiformis* have $2n = 24$, while *C. sicyoides* $2n = 48$ chromosomes.

Genus *Tetrastigma* also displays various chromosome numbers, $2n = 22$, 44, or 52. According to Gamble and Lawson (cited in Shetty) and, in particular, to Shetty (1959), who undertook a comparative study of the chromosome morphology of several *Tetrastigma* species, the genus seems to be a homogeneous one.

Cayratia species again are reported in the literature to have various chromosome numbers, namely $2n = 30$, 60, 72, or 98. Shetty (1959), upon carrying out a comparative caryological analysis of three *Cayratia* species, gives reason to botanists (Jussieu, 1823; Gagnepain, 1911; Gamble, 1918), who grouped under this denomination several species formerly placed in other Vitaceae genera (e.g. *Vitis*, *Cissus*) in order to constitute an independent genus. He also stated that, notwithstanding the differing chromosome number and its non-uniformity, the *Cayratia* caryotype cannot be paralleled to that of genus *Vitis* or genus *Cissus*, therefore the respective species build together a more or less homogeneous genus.

Upon having ascertained the chromosome number in 27 taxonomic units belonging to 6 genera and upon comparing our results with the available data in the literature, we feel able to state that genera *Vitis*, *Ampelopsis*, *Parthenocissus*, and *Tetrastigma* are homogeneous, unitary, well-defined genera.

However, since heterogeneous species were grouped into the genus *Cayratia*, based upon the caryological examination of only three of its species, we consider Shetty's statement that it is a homogeneous genus as a premature conclusion, though we agree these species must be separated from the genera *Vitis* and *Cissus*.

Our morpho-palynological studies enabled us to examine from this particular standpoint a bigger number of taxonomic units than that we succeeded to analyse caryologically and embryologically.

The morpho-palynological data we obtained bear out both the conception on the unity of the Rhamnaceae family and the notion of separating from the Vitaceae the genus *Leea* in order to build up an independent family, while inside the Vitaceae data collected from species belonging to 9 genera enabled some considerations on the unity of the respective genera and on their mutual relationships.

The morpho-palynological data we described in 20 *Cissus* species corroborate Shetty's statement about the unity and homogeneity of this genus. In 19 species investigated the pollen grains have most properties in common, the differences between the species being restricted to differences in length of the colpi and in slight morphological details, such as rounded or sharply ending shapes, the varying thickness of the fold bordering the colpi, the presence or absence of a crest around the pores, and the pili length ratio or pili capita size. Among the 20 *Cissus* species examined, *C. assamica* alone was found to differ morphologically, namely in the fact its pollen grains display a simply tegillate-baculate exine structure, while pollen grains of the 19 other species had a pilate exine in optical section. This species was attributed in the past to various Vitaceae genera: *Vitis assamica* Laws., *V. assamica* Fl., *Ampelopsis assamica* (?), and, in Merrill and Perry, 1941, *Cissus assamica* (Laws.) Craib.

About the unity of genus *Tetrastigma* only positive statements were published (Gamble, Lawson, Shetty). This opinion is in fact corroborated by such morphological features of the pollen grains as: their uniform size, the fineness of the sporoderm, the sporoderm's structure in optical section, the shape and the size of the colpi and the colour of the pollen grains.

Genus *Parthenocissus*, from a caryological standpoint welldefined, is palynologically too a well-individualized genus: it has medium size pollen grains; rounded triangular ambitus; the colpi length equals $\frac{2}{3}$ of the grain radius, the colpi being always terminally acute, delimited by a deep fold formed by rolled-up exine; the sporoderm surface is ornate, in optical section pilate-sympilate, the various species differing in the degree of thickening of the intine at the level of the pores.

The morphological structure features of the *Ampelocissus* species investigated also indicate a homogeneous, well-defined genus, namely:

in all species examined the pollen grains are small, with narrow, more or less short, terminally rounded colpi, the sporoderm surface is reticulated, while its structure in optical section is pilate-sympilate. The species in this genus can be distinguished from one another by the shape of the reticulum on the sporoderm surface and, in particular, by the thickness of the intine at the level of both mesocolpi and colpi.

The above considered genera *Ampelocissus*, *Cissus*, *Parthenocissus*, and *Tetrastigma* are well-defined from a morpho-palynological point of view; they build distinct unitary genera within the Vitaceae family.

The structural properties of pollen grains from various species of the other Vitaceae genera denote the existence within each of them of several sections or series.

By some of the morphological characters of the pollen grains, such as size and epistucture of the reticulated sporoderm, genus *Ampelopsis* appears to be a homogeneous, unitary genus. However, based upon the optical section structure of the sporoderm and upon the length of the colpi, we can distinguish here two series, namely a series with short colpi and with a pilate-sympilate optical section structure of the sporoderm, which will include *A. aconitifolia*, *A. heterophylla*, *A. brevipedunculata*, *A. japonica*, and a second series, in which the ridges of the meshes possess a simply tegillate-baculate structure, and this will include *A. cantoniensis*, *A. delavayana*, *A. javanica*, *A. leoides*. The first series is very closely related to genus *Vitis*, while the second one is closer to genus *Cayratia*.

Among the morphological features of pollen grains in *Pterisanthes* species which support the unity of this genus we can enumerate: the small size and the spheroidal shape of the pollen grains, the terminally sharpened colpi and the fineness of the sporoderm; the species are distinguished by the fact *Pt. coriacea* and *Pt. cissoides* display a sporoderm epistucture of reticulate appearance and in optical section a pilate structure, while *Pt. polita* displays a reticulated sporoderm epistucture and in optical section a pilate-sympilate sporoderm structure; therefore, genera *Pterisanthes* according to the morphological characters of the pollen grains the latter will be situated between genera *Vitis* and *Cissus*.

Genus *Rhoicissus*, by the morpho-palynological characters of its species, is closely related to the genera *Parthenocissus*, *Ampelocissus*, and *Tetrastigma*. One species, *Rh. thunbergii*, has acolporate pollen grains with an ornate sporoderm surface. So far, acolpia was not reported in the Rhamnales Order, which engenders some doubt as to whether the species was correctly placed here or else whether the herbarium specimen, our palynological material was sampled from, was correctly determined.

Genus *Vitis* displays distinct uniformity with respect to the morphological characters of the pollen grains. Taking into account the sporoderm epistucture, as well as its optical section structure, one can distinguish two groups, namely: the *Vitis silvestris*-type group, including most of the species we investigated, with an epistucture of reticulate appearance, built up by a fine network, to which a pilate exine structure corresponds in the optical section, with long pili and small capita (the

fineness of the structure renders the distinction of the species very difficult), then the second group, the *V. labrusca*-type group, including a smaller number of species, displaying pollen grains with a laxer network as an epistruature, either reticulated or — more frequently — ornate, while the optical section structure of the sporoderm is pilate to sympilate. Interspecific differences are found in each group in the length of the colpi, in the thickness of the sporoderm, in the existence or absence of a small crest around the pores.

The morpho-palynological data we obtained on the pollen grains of the *Cayratia* species investigated, such as the narrowness of the colpi, their lack of neat limits, the frequent presence of a continuous ring around the irregularly outlined pores, — all morphological features not encountered in the other genera of family Vitaceae, justify the shifting of these species from genera *Cissus*, *Ampelopsis* and *Vitis* into this genus. However, the other morpho-palynological properties, so diverse, are an indication *Cayratia* is a heterogeneous genus. Thus, for instance: the circular or rounded or (more or less) lobed triangular ambitus; the sporoderm thickness varying between 0.75 and 2 μ ; the sporoderm epistruature which may be of reticulate appearance, or reticulated (uniformly, or more often non-uniformly), or again ornate; the optical section structure of the sporoderm which may be pilate, pilate-sympilate or simply tegillate-baculate; the intine, which displays great variability as to its thickness at the level of the pores; the presence of syncolpate pollen grains in *C. mollissima*. This genus is unique in the Vitaceae family in displaying such a diversity of morpho-palynological characters and only extensive caryological, morpho-palynological and embryological studies in the majority of the species included in this genus will enable an elucidation of their exact taxonomic value.

Thus, taking into account the complexity of the morphological characters of the pollen grains and, in particular, the optical section structure of the sporoderm, we are able to find out relationships between the genera or between species groups within a given genus, according to structural affinity.

A first group, characterized by the pilate structure of the sporoderm, can be made out of the genera *Cissus*, *Pterisanthes*, and the *V. silvestris* section of genus *Vitis*.

A second group, characterized by the pilate-sympilate optical section structure of the sporoderm, can be constituted from genera *Ampelocissus*, *Tetrastigma*, *Rhoicissus*, *Parthenocissus*, and the first section of genus *Ampelopsis* and the *V. labrusca* section in genus *Vitis*.

A third group can be formed from the second section of genus *Ampelopsis*, as described above, displaying a simple tegillate-baculate optical section structure of the sporoderm.

Between these three groups lies genus *Cayratia*, with its remarkable diversity of morphological features of the pollen grain structure.

This grouping, based upon the morphological characters of pollen grains alone, has, of course, only an indicative value, since "no character is able by itself to give the key to the natural group of the Ampelideae" (Planchon, 1887).

M. Reille (1967), in his 'Contributions to the palynological study of the family Vitaceae' studied 52 species belonging to 9 genera; only 10 species therein coincide with ten among the 112 species belonging to 9 genera of the same family we investigated. Among the genera dealt with by M. Reille only genus *Cyphostemma* was not included in our work, while we studied *Pterisanthes* species, a genus missing in M. Reille's report. Also, he used Faegri and Iversen's terminology, while we chose to use that worked out by Erdtman (1952). M. Reille considers the essential feature of the sporoderm optical section structure in Family Vitaceae to be the simply baculate muricate exine displaying complex muri. In our studies we found that the exine may have either a pilate structure, such as occurring in genera *Pterisanthes* and *Cissus*, in many species of genus *Vitis* and in some *Cayratia* species (in the latter instance the sporoderm surface has a reticulate appearance, though no true reticulum is available), or a pilate-sympilate structure, such as occurring in members of the genera *Ampelocissus*, *Tetrastigma*, *Parthenocissus*, and *Rhoicissus*; furthermore, only in a few species the structure is a simply tegillate-baculate one at the level of the walls delimiting the meshes of the network (and in such case the epistucture of the sporoderm is reticulated or ornate): this structure type is encountered in some species belonging to the genera *Ampelopsis* and *Cayratia*, as well as in *Cissus assamica*.

Though most of the material dealt with is different, as well as the terminology used and the results obtained concerning the sporoderm optical section structure, and although M. Reille formulates some general considerations based upon the morphological characters of the colpi and pores, besides those upon the sporoderm structure, while we particularly took into account the optical section structure of the sporoderm, the conclusions as to the relationships between the families within the Rhamnales Order, as well as to the value or connections between the genera in the Vitaceae family are approximately similar. The main difference would lie in the fact that author considers *Cayratia* a homogeneous and unitary genus, while we do not share his view.

The morphological peculiarities of the microsporogenesis process in the 12 Vitaceae taxonomic units investigated confirmed the literature data regarding the normal run of this process in most of the taxonomic units examined, both with respect to the anther structure and to the effectuation of microsporogenesis (Baranov, 1946; Mulay, Nair and Sastry, 1953; Nair and Bajaj, 1966).

Our results also confirmed Baranov's assertions (1946) concerning the sterility encountered in the grapevine, which is due to anomalies occurring in the microsporogenesis process, namely: a cessation of the nucleus divisions in the pollinic mother cell, usually happening in the binucleate stage (in female flowers in *Vitis silvestris* and in the Rumanian grape variety 'Crîmpoşie') or the formation of non viable microspores (pollen grains) devoid of germinative pores.

The examination of the male gametophyte development in 20 taxonomic units of the family Vitaceae led us to results confirming the data published in the literature (Dorsey, 1914; Suessenguth, 1935; Baranov,

1946 ; Mulay, Nair and Sastry, 1953 ; Schultze-Motel, 1964), namely the fact bi-trinucleate pollen grains are characteristic for this family, the particular feature of the pollen grains germinating in the vesicle (Baranov : in *Vitis vinifera* and in some Asian cultural varieties). For the first time in these taxonomic units the progress of the nuclei inside the pollinic tube was established, as well as the site where the generative nucleus divides, the time period required for the complete formation of the pollinic tube in various culture media and, finally, similarities or differences in this respect between the taxonomic units investigated.

In the available literature we could not find any report on polysiphonism with members of this family, though we met such in our investigations in *Parthenocissus quinquefolia*, *Vitis longifolia* and in the Rumanian grapevine cultural varieties 'Gordin', 'Plăvaie', and 'Galbenă de Odobești'.

The study on the reproductive sphere and, in particular, on the development of the female gametophyte in 11 taxonomic units of the family Vitaceae and in one *Leea* species corroborated the morpho-palynological data in demonstrating the unity of family Vitaceae and the fitness of the notion of separating genus *Leea* from the Vitaceae genera and of raising it to the rank of a family within the Rhamnales Order.

The comparative analysis of the morphological features of the female gametophyte in the various taxonomic units examined belonging to genera of the family Vitaceae also support the conclusions drawn from caryological and morpho-palynological data as to the relationships between these genera.

The data obtained mostly confirm the reports in the literature on morphological features of the reproductive sphere and, in particular, those of the female gametophyte in the Vitaceae, as concerning the ovule type, the presence of calottes (both nucellar and epidermal), the type of the embryo-sac, the type of fecundation, etc.

All the investigators who studied the female gametophyte of the Vitaceae (Baranov, 1946, in *Vitis vinifera* and in some of its Asian cultural varieties ; Mulay, Nair and Sastry, 1953, in *Vitis pedata* ; Nair and Bajaj, 1966, in *Cyphostemma setosum* ; Dvornic, 1961, in the grapevine variety 'Hamburg Muscatel') state that the antipodes get disorganized before the fecundation process. In contrast with this we observed in all instances the presence of antipodes both during the fecundation process and afterwards — truly not without some difficulty, since they are lying in a fine haustorial extension of the embryo-sac, immediately close to the chalaza. With most of the taxonomic units investigated the three antipodes persist during fecundation, except in *Parthenocissus quinquefolia* and in the grapevine variety 'Pinot gris', where one antipode is resorbed so that only two can be detected, then in *Cayratia pedata* and in *Ampelocissus thyrsoflora*, where the antipodes multiply, so that subsequent to the fecundation six antipodes are detected. Independently of their number, their volume increases during the fecundation

process, they get wrapped in a manifest plasmatic film and eventually display a spherical or pyriform outline.

The porogamous type of fecundation, characteristic for the Vitaceae, is also confirmed with most of the taxonomic units analysed, except in *Ampelopsis heterophylla*, where the pollinic tube penetrating the ovule stalk enters through the integument apex into the nucellus, and in *Cissus assamica*, where the embryo-sac — its apical head first — gets across the nucellus and, passing through the micropyle, reaches immediately close to the ovary wall, while the pollinic tube penetrates directly from the ovary wall into embryo-sac.

A quite particular instance, not yet reported as a characteristic feature of the Vitaceae, is represented by the female gametophyte development in *Cissus* species, where, during its development, the mother cell of the embryo-sac consumes the three apical macrospores, the nucellar and the epidermal calotte, and the inner part of the interior integument bordering the micropyle, so that the mature embryo-sac will leave the nucellus with the oosphere apparatus part and reach the close vicinity of the ovary wall, whose cells have a papillary shape and are located very near to the apical area of the ovule, which eventually renders the ovarian cavity almost inexistent.

From a morpho-palynological point of view, this species lies nearer to the *Ampelopsis* species group characterized by a simply tegillate-baculate sporoderm in optical section structure, than to *Cissus* species; from an embryological standpoint it differs from all other species investigated. In the systematics of the Vitaceae family this species was variously placed: *Vitis assamica* Laws., *V. assamica* Fl., *Cissus adnata* Roxb. (King, 1896), *Ampelopsis assamica* (?), and, last to date it was mentioned in Merrill and Perry's work (1941) as *Cissus assamica* (Laws.) Craib.

In our opinion, it was correctly placed into genus *Cissus*, particularly in consideration of the flower morphology, namely with respect to the sub-terminal mode of detachment of the petals. Taking into account the peculiar features of the female gametophyte development in this species by comparison with the other genera to which it was ascribed, it is obvious it cannot be included either in genus *Vitis* or in genus *Ampelopsis*.

The other *Cissus* species we investigated: *C. discolor* and *C. cactiformis*, by the anomalies they display do not enable any comparison with *C. assamica* with respect to the details of the female gametophyte development. Only the extension of embryological studies to other *Cissus* species will help to elucidate the point whether *Cissus assamica* is to be included in this genus or elsewhere.

By comparing the particular features of the female gametophyte development in *Cayratia pedata* (Lam.) Juss., as resulting from our analysis, with those published by Mulay, Nair and Sastry (1953) for *Vitis pedata* (?) we reach to the conclusion it is the same species. Though the investigators do not mention the author's name, we think they worked on *Vitis pedata* Valh. ex Wall., cited in Quisumbing's paper (1944) as synonymous to *Cayratia pedata* (Lam.) Juss.

We consider this species is well placed in the genus *Cayratia* and cannot be maintained in genus *Vitis*, in consideration of both its morpho-palynological and embryological characters, among which we would point out the antipode multiplication subsequent to the fecundation process. The authors mentioned above state — along with others having dealt with the embryology of the Vitaceae family — that antipodes get disorganized prior to the fecundation process, a fact we could not confirm in our investigations.

It was affirmed *Cissus discolor* does not form any seeds (respectively, fruit), but gets multiplied only vegetatively, though not specifying the causes of this sterility. In the analyses we carried out, we found both with *C. discolor* and with *C. cactiformis* (in material sampled in the greenhouse) that their sterility is due to a stagnation of the embryo-sac development at the stage of 2—4 even 8 nuclei, where however the eight nuclei do not organize in the oosphere apparatus, the antipodial apparatus and the secondary nucleus of the embryo-sac. The whole ovule body has an abnormal development: the ovule stalk is long, the nucellus restricted, the outer (in *C. discolor*) or both integuments (in *C. cactiformis*) display a remarkable growth (depending on the length of the ovule stalk), thus delimiting the long micropyle. Above the nucellus the inner integument cells are often disorganized, letting a free space occur.

It has been remarked in the literature that very few seeds are formed in *Parthenocissus quinquefolia*, out of proportion with the big number of flowers. In our embryological study we found this phenomenon is due to the restricted germinative power of its pollen grains. This pollen germinates in a proportion of 80%, out of which only 15% build normal pollinic tubes, while the remaining, whilst attaining more or less the same length, are abnormal. The strong proportion of abnormal pollinic tubes is to be ascribed, in our opinion, to the fact the pollinic tube grows at the expense of the plasma, while the two nuclei do not leave the pollen grains; as well as to the fact the nuclei progression occurs in a chaotic way. These anomalies are also encountered in other species belonging to the Vitaceae, however, in none of them to such an extent as in *Parthenocissus quinquefolia*.

The available literature does not contain any data on the time required, starting from the moment of pollenization, for the pollen grain germination, for the growth of the pollinic tubes, and for the fecundation process in members of the Vitaceae family, except for Baranov's statement (1946) that "the effects of fecundation are seen in the grapevine as early as the following day after pollenization in the considerable increase of the ovary volume".

We carried out artificial pollenization tests in *Vitis silvestris* and in the grapevine varieties 'Pinot gris' and 'Gordin', and we found differing results: the fecundation process took in *Vitis silvestris* 60 minutes, while in the two cultural varieties 15 minutes only.

Summarizing the investigations described above, we can state as follows:

The caryological study performed in 20 taxonomic units of the Vitaceae family confirmed the literature data concerning 12 of them and ascertained for the first time the chromosome number in 8 taxonomic units, besides enabling some evaluation of the unity or of the mutual relationships of the genera involved.

The morpho-palynological study done in 151 taxonomic units belonging to the three families in the Rhamnales Order: Rhamnaceae, Vitaceae and Leeaceae, contributed to a verification of the phylogenetic relationships between these families and between various genera within the family Vitaceae. The structure of pollen grains in the Rhamnaceae, in 30 *Leea* species and in 102 taxonomic units from the Vitaceae was described, about which no literature data were available.

The analysis of the male gametophyte development (in 20 taxonomic units of the family Vitaceae) and that of the female gametophyte development (in 11 taxonomic units of Vitaceae and in one *Leea* species) as the most conservative ones, by demonstrating their particular features have considerable significance for plant systematics and phylogeny: they enabled us to check the phylogenetic relationships between the families Vitaceae and Leeaceae, to make some comments on the systematic value of the genera included in the Vitaceae and of some species of uncertain systematic position.

Complex multilateral studies are necessary for the elucidation of phylogenetic relationships between the various plant groups, since no isolated character can be given an exclusive importance. The difficult systematics of the Vitaceae requires further caryological, morpho-palynological and, in particular, embryological studies in a large number of members in order to improve the actual delimitation of the genera.

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MACROSPOROGENEZA, DEZVOLTAREA GAMETOFITULUI FEMININ ȘI ÎNCEPUTUL DIFERENȚIERII EMBRIONULUI LA UNII REPREZENTANȚI AI FAMILIEI VITACEAE ȘI LEEACEAE, CU CONSIDERAȚIUNI FILOGENETICE

Re z u m a t

Contribuția de față cuprinde descrierea pentru prima dată a particularităților macrosporogenezei, a dezvoltării gametofitului feminin, a procesului de fecundare și a începutului diferențierii endospermului secundar de tip nuclear, precum și diferențierea embrionului la 11 taxoni din familia Vitaceae și 1 taxon din familia Leeaceae.

S-a stabilit pentru prima dată timpul de fecundare la *Vitis silvestris* și la 2 soiuri de cultură. De asemenea, contrar celor cunoscute din literatura de specialitate, s-a demonstrat existența antipodelor în și după fenomenul de fecundație, mărirea volumului lor și chiar multiplicarea lor.

Lucrarea cuprinde concluzii filogenetice asupra Ordinului Rhamnales, pe baza datelor cariologice, morfopalinologice și embriologice originale, corelate cu cele din literatura de specialitate.