Embryosac and embryo of Moringa oleifera Lam.: the female gametophyte of angiosperms

EMBRYOSAC AND EMBRYO OF MORINGA OLEIFERA LAM..

THE FEMALE GAMETOPHYTE OF ANGIOSPERMS.

Diss Utrecht-gu 1923

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PROEFSCHRIFT TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKS-UNIVERSITEIT TE UTRECHT OP GEZAG VAN DEN RECTOR MAGNIFICUS J. F. NIERMEYER, HOOGLEERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, VOLGENS BESLUIT VAN DEN SENAAT DER UNIVERSITEIT TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE TE VERDEDIGEN OP MAANDAG 22 JANUARI – 1923, DES NAMIDDAGS TE 4 UUR –

DOOR

FREDERIK LODEWIJK RUTGERS,

GEBOREN TE AMSTERDAM.

N. V. BOEKHANDEL EN DRUKKERIJ
VOORHEEN E. J. BRILL, LEIDEN
1923.

PARENTUM MEMORIAE.

Militaire verplichtingen hebben mijn studententijd, nu ruim acht jaren geleden, tot een abrupt einde gebracht, en langen tijd iederen regelmatigen arbeid belet. Later nam mijn maatschappelijke werkkring mij in steeds toenemende mate in beslag, zoodat het thans voltooide werk soms maanden achtereen moest blijven rusten. Slechts dan toch achtte ik mij gerechtigd daaraan te arbeiden, wanneer aan alle andere aanspraken op mijn werkkracht ten volle was voldaan. Het late verschijnen van dit proefschrift moge hiermede gerechtvaardigd zijn.

Dankbaar erken ik de groote waarde, die de academische vorming voor mij heeft gehad, en gedenk hierbij in de eerste plaats de hoogleeraren in de Wis- en Natuurkundige faculteit, wier onderwijs ik aan de Amsterdamsche Universiteit genoten heb. In het bijzonder U, Hooggeleerde Hugo de Vries, ben ik veel verschuldigd. Uw leiding, Uw belangstelling in mijn studie, en vooral ook het voorrecht, dat ik een tijdlang Uw assistent heb mogen zijn, worden door mij steeds in dankbare herinnering gehouden.

Hooggeleerde Went, het verheugt mij hier een gelegenheid te hebben openlijk te kunnen gedenken de bereidwilligheid, waarmede gij op het verzoek om als mijn promotor op te treden, zijt ingegaan; de hulpvaardigheid, waarmede gij mij het materiaal voor mijne onderzoekingen hebt verschaft; en de welwillendheid, waarmede gij mij bij de bewerking van dit proefschrift in alles zijt tegemoet gekomen. Voor Uw moeite, Uw tijd, Uw voorlichting en Uw belangstelling ben ik U blijvend erkentelijk.

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Reliquia Treubianae III.

Embryosac and Embryo of Moringa oleifera Lam.

Moringa oleifera Lam. (pterygosperma Gärtner) is the best known representative of the tropical family of the Moringaceae. Indigenous to the Indies the species has been cultivated for many thousand years and is to be found in African and American tropics as well as in Asia.

Material.

One of the very last studies published by TREUB (Le sac embryonnaire et l'embryon dans les Angiospermes. Nouvelle série de recherches — Ann. du Jard. Bot. de Buitenzorg 1910) was meant as the first of a series of articles on the subject. For this purpose he brought home an extensive material relating to embryosac formation in numerous tropical families. Afterwards the collection passed to Prof. WENT of Utrecht University who kindly put at my disposal the Moringa material collected by TREUB at Buitenzorg (Java), which was fixed in alcohol.

From still two other sources material was available at Utrecht. BOLDINGH collected on the isle of Curacao (Dutch West Indian archipelago) and KUYPER in Surinam (Dutch Guyana). Fixation by both in alcohol and in Flemming's.

During the examination it turned out that only *embryosac* formation could be studied from the material at hand. Even in seeds of considerable size no trace of *embryo* formation was seen, though full-grown seeds were known to carry quite normall embryo's. Dr. STAHEL at Paramaribo (Surinam) kindly helped me by sending additional stadia, collected by him and fixed in alcohol, which fixation proved itself to be the best.

Though of different origin the material was of absolute uniformity as to megaspore- and embryosac formation. KUYPER's material however showed some delay in development. Ovaries of a size in which usually mature sacs are found, contained but tetrads or exceptionally a two-nucleate stage. This delay is distinct from the beginning, the nucellus remaining without any differentiation until an exceedingly advanced (Plate I, Fig. 3) and far later stage than usual in all other material.

The examination of many thousands of sacs underlies this publication, but never anything has been seen, which was not in conformity with the following description of the development of the female gametophyte.

The Megaspores.

Ordinarily the archesporium is distinguishable about the time of the first differentiation of the integuments. It is onecelled and hardly to be recognised from the cells of the surrounding nucellar tissue. (Plate I, Fig. 1). Occassionally a two celled archesporium is met with (Plate I, Fig. 2), both cells showing the same germinating capacities, which might lead to two tetrads and even to two complete embryosacs lying paralel.

The archespore cell does not divide and is the embryosac mothercell (Plate I, Fig. 4). Parietal tissue is totally suppressed, but exceptionally archespore cells containing two nuclei are met with. (Plate I, Fig. 5, 6). In no case were cell walls seen.

The embryosac mothercell gives rise to two cells of unequal size, a large inner one and a small outer cell (Plate I, Fig 7). In the second division the spindle of the outer cell lies rectangular to the axis of the sporangium. Thus the four megaspores are never found in a row, but always the two outer cells at a right angle with the inner ones (Plate I, Fig. 8).

Megaspore formation is followed by a rapid desintegration and final disappearance of the outer cells. The first signs of destruction are already seen when the functioning inner one is still one-nucleate and has hardly begun to grow (Plate I, Fig. 9) and even before the end of the two-nucleate stage has been reached their disappearance is complete (Plate I, Fig. 12, Plate II, Fig. 13).

The Embryosac.

The inner megaspore which from the very first moment shows itself the functioning one, is regularly filled up with cytoplasm. Its nucleus is to be found at the top end, where it divides (Plate I, Fig. 10, 11). Soon after this first division one of the daughter nuclei commences to move to the lower end of the sac. This migration is accompanied by an ever increasing polarisation, the result being the typical and well know figure of the polisared two-nucleate stage of the embryosac: two nuclei separated by a large central vacuole (Plate I, Fig. 12, Plate II, Fig. 13, 14), and both embedded in a comparatively small mass of cytoplasm.

By a second division the four-nucleate stage is reached. (Plate II, Fig. 18, 19). The primary micropylar nucleus however seems to be in advance (Plate II, Fig. 15), sometimes even as much as having finished its division when the primary chalazal nucleus is still at rest (Plate II, Fig. 16, 17). Embryosacs are then three-nucleate.

The four-nucleate stage is followed by a division of one of the nuclei only, both chalazal and one micropylar nucleus remaining undivided. So at the top end never more than three and at the chalazal end never more than two nuclei are seen. (Plate III, Fig. 20, 21, 22). The egg apparatus is formed in the usual way, the three nuclei get separated by cell walls (Plate III, Fig. 23) and finally the two well shaped synergids partly cover the egg (Plate III, Fig. 24). In the mature sac cytoplasm in the cells of the egg apparatus shows the normal distribution. As usual the synergids are characterized by a large vacuole at the lower end, cytoplasm and nuclei being gathered at the topend.

During the formation of the egg apparatus the two chalazal nuclei are seen in close connection and steadily moving upwards (Plate III, Fig. 21, 22, 23). Finally a position is reached at the very topend of the embryosac quite close to egg and synergids (Plate III, Fig. 24). There has never been seen any sign of further division or of fusion.

Thus the mature embryosac is five-nucleate, three of the nuclei being of micropylar and two of chalazal origin. The

synergids are sisters, and an upper polar nucleus is lacking. Two chalazal nuclei have taken the position of an embryosac nucleus, and antipodals are missing.

The Endosperm and the Embryo.

Fertilization takes place in a normal way, the pollentube discharging its contents in one of the synergids (Plate III, Fig. 25). One male nucleus fuses with the egg nucleus and the second male nucleus moves towards the "embryosac nucleus" (Plate III, Fig. 26). Up to this very moment the latter has retained its double character and so three nuclei are seen fusing, giving rise to the primary endosperm nucleus.

Endosperm formation commences at once and soon a great many nuclei are present. They are especially numerous at the top end and some are found all along the walls of the embryosac. All the time the sac is rapidly increasing in size, but the fertilized egg shows no signs of any activity. Not until the seed has reached a length of about 4 mm. does the egg's first division take place (Plate VI, Fig. 35: mature sac — and sac after second division of egg).

Embryo development begins with free nuclear division. The egg nucleus divides without cell wall formation giving rise to a two-nucleate embryo (Plate IV, Fig. 27). This first division is followed by a simultaneous division of both nuclei, their spindles at a right angle. The four nuclei resulting from this division are not lying on the same level. Somitimes they are found in two succesive sections (Plate IV, Fig. 28) and when in one figure (Plate V, Fig. 29) they are still distinctly on separate levels. The free nuclear division goes on till a sixteen-nucleate embryo stage is reached (Plate V, Fig. 30). Then walls are formed, the 16-nucleate embryo thus developing into a 16-celled one (Plate V, Fig. 31). Simultaneous divisions have come to an end now, seven of the sixteen cells figured, containing one nucleus each, and eleven of them showing two nuclei.

The endosperm is still without cell walls, the free nuclei lying embedded in a common mass of cytoplasm (Plate VI, Fig. 33, 34). Cytoplasm however becomes more and more vacuo-

lated and shortly afterwards is found divided by cell walls. (Plate VI, Fig. 32). At this stage seeds have already attained a length of about 8 mm. and the fruits even of 15 to 25 cM.

Summary.

The archesporium of Moringa oleifera Lam. consists of one single cell.

Four megaspores are formed, the outer two lying rectangular to the axis of the sporangium.

The inner megaspore is the functioning one. By two succesive divisions the normal polarised four-nucleate stage is reached. The third division is restricted to one of the micropylar nuclei only, the other micropylar and both chalazal nuclei remaining undivided.

Thus the mature embryosac is five-nucleate, showing a normal egg apparatus of two synergids (sisters) and the egg. The position of the embryosac nucleus is taken by the two chalazal nuclei, lying in close contact and quite near the egg.

At fertilization one male nucleus fuses with the eggnucleus and the other male nucleus enters in triple fusion with the chalazal nuclei. This primary endosperm nucleus at once commences to divide and soon numerous free endosperm nuclei are present. The egg remains undivided for quite a long time.

Embryo formation begins with free nuclear division up to the sixteen-nucleate stage. Then cell walls are formed and further divisions no longer take place simultaneously. The embryo rapidly grows and the cytoplasm in which the endosperm nuclei are embedded is divided by cell walls.

This way of megaspore — and of embryosac formation, leading to a 5-nucleate sac of a very special character, up till now has only been reported for Garcinia (M. Treub: Ann. du Jard. Bot. de Buitenzorg 1910: Le sac embryonnaire et l'embryon dans les Angiospermes. Nouvelle série de recherches, I Garcinia Kydia (Roxb.), Garcinia Treubii (Pierre)).

The female gametophyte of Angiosperms.

I. INTRODUCTION.

Up to the beginning of this century hardly any attention was paid to the study of the development of the Angiosperm embryosac. Only very few cases of "abnormal sacs" being known, it was generally accepted that the development was of a most striking uniformity throughout the whole group. This normal course runs as follow: By two successive divisions the embryosac-mothercell gives rise to a row of four cells, called megaspores. Three of these soon begin to degenerate while the fourth, rapidly increasing in size, becomes the functioning embryosac. Its nucleus divides itself thrice thus producing eight nuclei, originally free in the same plasm but soon separated by cell-walls. These eight nuclei are arranged into two groups: viz. a micropylar one (egg and two synergids) and a chalazal one (three antipodals). In the middle of the sac the two remaining nuclei (polar nuclei) are seen fusing (embryosac-nucleus).

The last fifteen years however have brought to light an ever growing number of atypically developing sacs. Deviations in almost every direction were detected. First of all the number of megaspores seems to vary from the normal four to only one ("row of three", "row of two" or "embryosac-mothercell functioning as embryosac"). Secondly the number of nuclei in the fullgrown sac is far from being regular. Instead of the usual eight there may be sixteen or only four; not to speak of the numerous cases in which a secondary increase or decrease of nuclei could be stated.

Some of these pecularities are characteristic to certain spe-

cies or families, while others affect representatives of widely separated groups. Very soon the question rose whether these irregularities ought to be considered as more primitive or as more advanced than the normal type. The origin of the Angiosperm embryosac is still utterly unknown and it was hoped that some light might be given by the study of "abnormal sacs". So gradually the attention became focussed on the problem: how to arrange the deviations from the normal type in a useful system.

If only a survey of the material was wanted we should be free to choose one or two of the most prominent characteristics as a base on which a scheme could be built. Since however an insight in phylogenetical problems is wanted, we are bound to reckon with *all* the facts that coöperate in embryosac-formation. There can be no objection to stating that artificial systems are entirely worthless for phylogenetical purposes. A useful system of the gametophyte necessarily must be *natural*.

We will try to give a complete survey of all atypical embryosacs and of the various systems in which they are classed by different authors. At the same time we will have full opportunity of discussing any questions of homologizing of the nuclei in the embryosac.

It is not the intention to give anything but the outlines of a natural system. Our knowledge of embryosac-formation in most families is still too defective to enter into particulars. Therefore those facts of which the systematical value is clear and which do not present any points for divergence of opinion, are only mentioned without further dicussion. So for instance whether the arrangement of the megaspore cells is ⋮ or ⁖ or ∴, whether the inner one or any of the other three becomes the functioning megaspore, whether one or more of these megaspore cells develop, etc. are details of systematical interest, for which reference to special studies on these subjects will answer.

II. LITERATURE.

It would bring us too far to discuss all literature on the origin of the Angiosperm embryosac. We will confine therefore

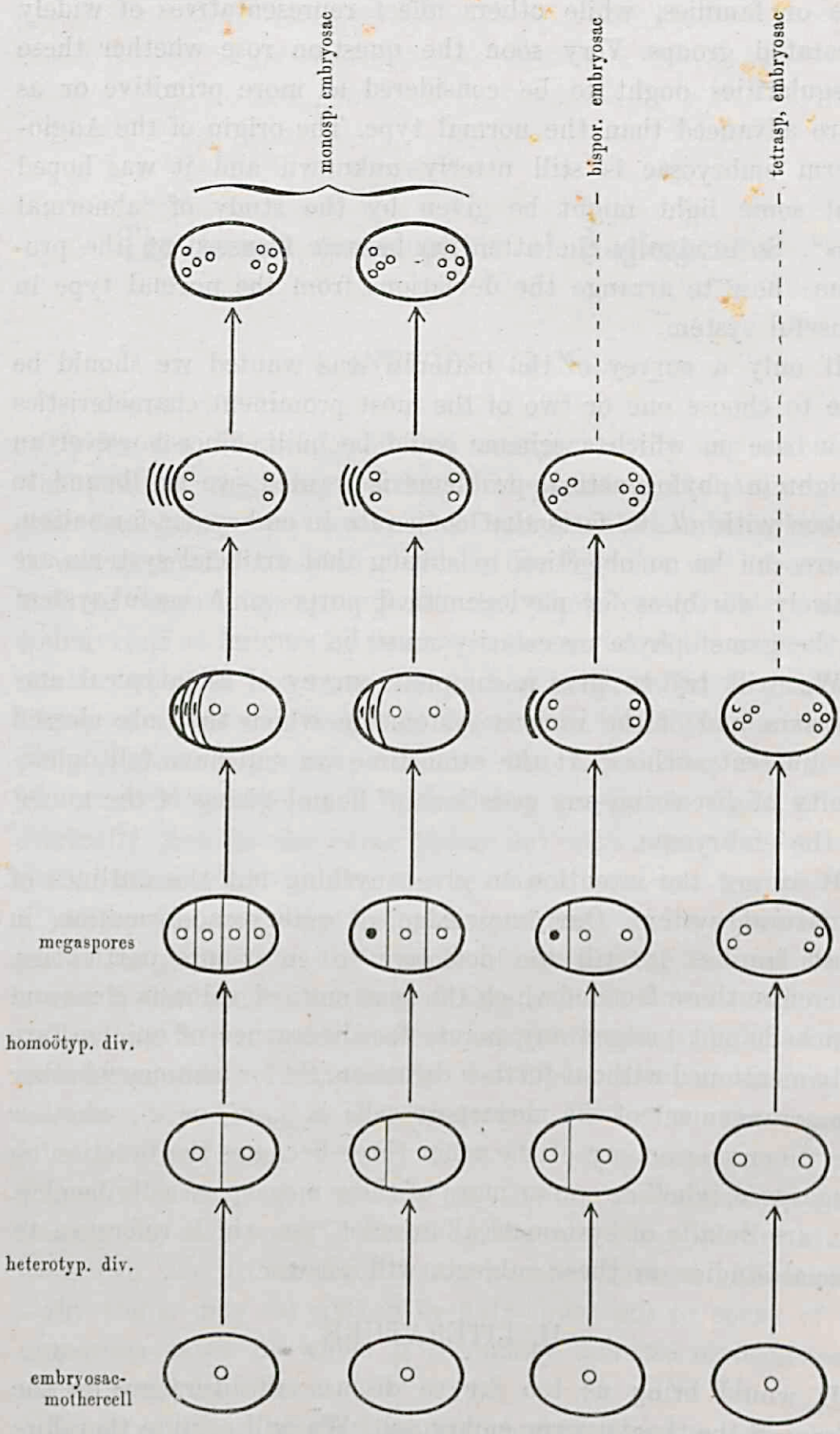

Fig. 1a. Coulter's conception of homologies in Angiosperm embryosacs.

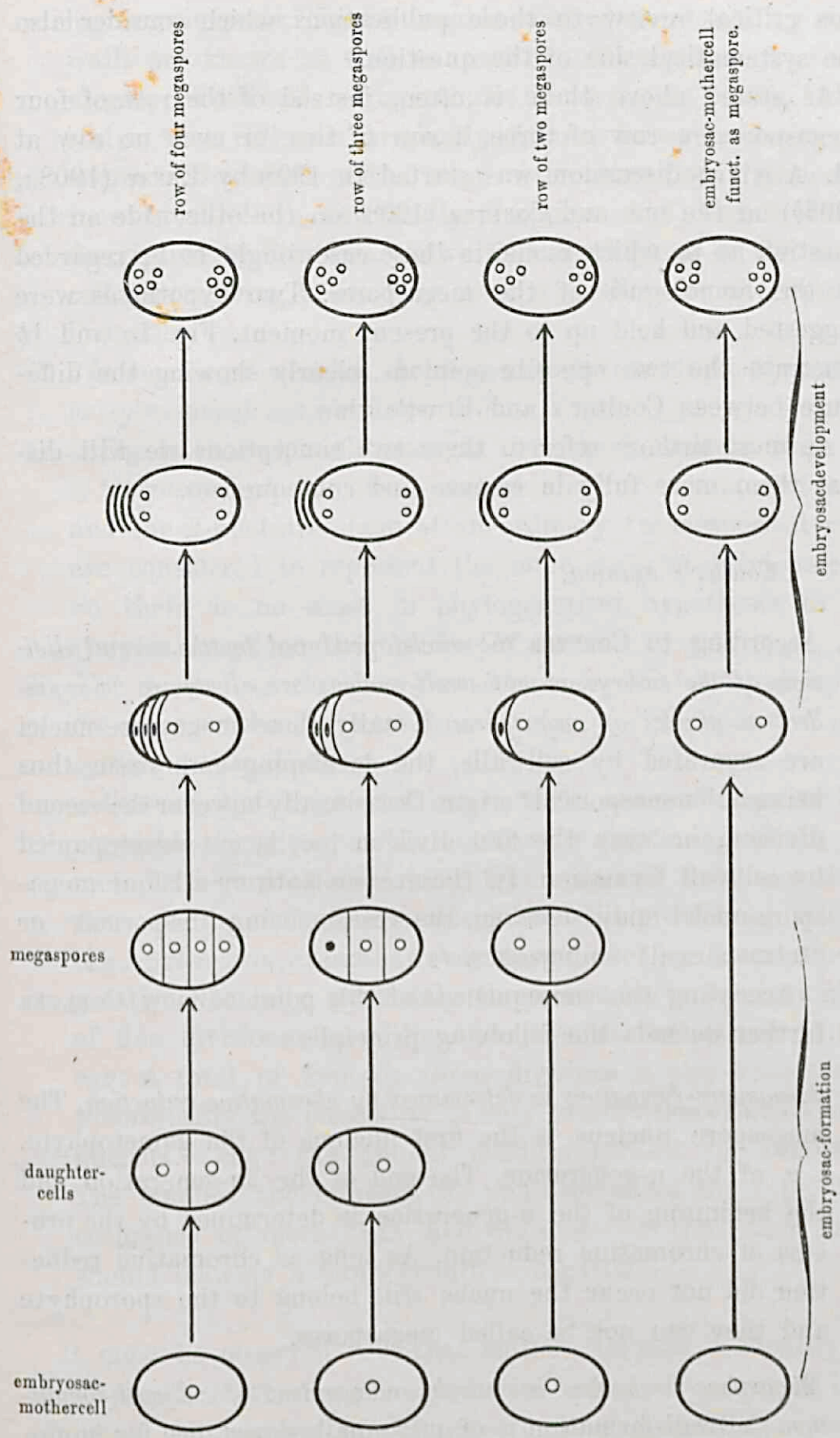

Fig. 1b. Ernst's conception of homologies in Angiosperm embryosacs.

this critical review to those publications which consider also the systematical side of the question.

As stated above there is often, instead of the row of four megaspores, a row of three, a row of two, or even no row at all. A vivid discussion was started in 1908 by Ernst (1908*a*, 1908*b*) on the one and Coulter (1908) on the other side on the question as to which nuclei in these cases ought to be regarded as the homologues of the megaspores. Two hypotheses were suggested and held up to the present moment. Fig. 1*a* and 1*b* illustrate the two opposite opinions, clearly showing the difference between Coulter's and Ernst's view.

As most authors refer to these two conceptions we will discuss them more fully in essence and consequences.

Coulter's opinion.

1°. According to Coulter *the nuclei produced by the second division of the embryosac-mothercell-nucleus are always to be regarded as nuclei of megaspores.* Usually these megaspore-nuclei are separated by cellwalls, the developing embryosac thus being of "monosporical" origin. Occasionally however the second division, or even the first division too, is not accompanied by cellwall formation. In these cases both or all four megaspore-nuclei may develop, the results being "bisporical" or "tetrasporical" embryosacs.

Accepting the consequences of this point of view, Coulter further defends the following principles:

2°. *Megaspore-formation is determined by chromatine-reduction.* The megaspore nucleus is the first nucleus of the gametophyte, i. e. of the n-generation. The end of the 2n-generation and the beginning of the n-generation is determined by the process of chromatine reduction. As long as chromatine reduction did not occur the nuclei still belong to the sporophyte and they can not be called megaspores.

3°. *Megaspore-formation is entirely independent of cellwall-formation.* Cellwall-formation is of only small importance for homo-

logising purposes. Unquestionable cases of desintegrating cellwalls are known as well as cases of total suppression of the megaspore-cellwalls. For instance Mc ALLISTER (1909, 1914) states that cellwalls originally formed between the megaspores of Smilacina and of some of the other Convallariaceae, soon break down and finally disappear. And SMITH's (1911) description of Clintonia shows four megaspores in the same cell, there being never more than a small trace of cellplate-formation.

4°. *The number of nuclei in the fullgrown sac has no phylogenetical or systematical significance at all.*

Fig. 1*a* shows plainly enought that the 8-nucleate stage of a "tetrasporical sac", the 4-nucleate stage of a "bisporical sac", and the 2-nucleate stage of an ordinary "monosporical sac" are considered to represent the same stage of development. So there is no sense in phylogenetical hypotheses or in systems, based on the number of nuclei.

5°. *The number of divisions from the embryosac-mothercell up to the egg furnishes the most valuable data for phylogenetical and systematical studies.*

One of the most striking facts in the evolution of the vegetable kingdom is the continuous shortening of the gametophyte-generation. Among Gymnosperms numerous divisions of the megaspore-nucleus are still prevailing. Within the Angiosperm group however this number has been reduced greatly. Normally it is only three, making a total number of five divisions from the embryosac-mothercell up to the egg. A total of four or three divisions is also known and theoretically the possibility of only two divisions (megaspore-nucleus = egg: the animal condition) must be admitted. The smaller the number the more advanced the type. The occurence of more than five divisions on the other hand should indicate a more primitive condition.

It must be observed here that neither the idea of identifying megaspore-formation with chromatine-reduction, nor the idea of using the number of divisions for phylogenetical purposes,

were new at the time of COULTER's publication. Miss PACE (1907) in her study on Cyprepedium pointed out how the embryosac with its three divisions between mothercell and egg, was well on the way to the animal condition. And the coupling of megaspore-formation to chromatine-reduction is yet met with in SCHNIEWIND-THIES' (1901), and propagated by DAVIS (1905), CHAMBERLAIN (1905), and PACE (1907). In fact all the ideas more fully developed by COULTER were already underlying Miss PACE's publication. She made exactly the same homologies and even saw the necessity of discerning mono-, bi- and tetrasporical sacs.

After COULTER's lucid statement most authors who dealt with the subject accepted his views and propagated his opinion, among them STEPHENS (1909*a*, 1909*b*), MC ALLISTER (1909, 1914), PACE (1909), SMITH (1911), BROWN and SHARP (1911), SHARP (1912), DAHLGREN (1915), KUSANO (1915), PALM (1915), HÄUSER (1916) and ISHIKAWA (1918).

BROWN (1908, 1909) however did not wholly agree with COULTER. He admits neither chromatine-reduction nor cellwall-formation as a criterion for megaspore-formation. According to him the appearance of cell-plates in the spindle figures of the first divisions furnishes the only certain characteristic of megaspore-formation. As long as cell-plates are present we have to do with spore-formation; when they are lacking, spore-germination is going on. This conception however can be regarded as wholly miscarried since from several sides attention was called to the fact that cell-plate-formation may occur at any stage of the embryosac-development, and apparently without any connection to megaspore-formation.

Systems based on Coulter's principles.

DAHLGREN's (1915) attempt to outline a scheme which should embrace all cases of atypical embryosacs, is still rather primitive. He discerns four groups. The first one, showing five divisions between embryosac-mothercell and egg, is represented by the normal eight-nucleate sac. Next comes a group with only four divisions, including the 16-nucleate Penaeaceae, the 4-nucleate Onagraceae, as well as Clintonia, Codiaeum and Lawia.

Only three divisions are found in Podostemon, Dicraea, Cypripedium, Helosis and Statice. While the last group (two divisions = the animal condition) shows a reduction to the utmost, as seen in Plumbagella.

It will be noticed that this "system" is thoroughly one-sided. All possible stress is laid on the number of divisions, without giving any thought at all to the number, the arrangement, or the origin of the nuclei in the mature sac. Not even the origin of the sacs themselves is regarded, for representatives of COULTER's mono-, bi-, and tetrasporical sacs are readily joined in the same group. Of the naturalness and phylogenetical significance of a system like this nothing needs to be said.

SAMUELS' (1912) scheme is a little more advanced. He too accentuates the number of divisions and uses them as main line for his system. (To DAHLGREN's four groups he added a fifth, with six divisions and thus more primitive than the normal type. This new group however can be dropped safely, since DESSIATOFF's (1911) observation of a "monosporical" 16-nucleate Euphorbia proved to be wrong). As a new factor SAMUELS introduced a subdivision of the groups by means of the mono-, bi-, or tetrasporical character of the sacs. In his system not only the number of divisions, but also the origin of the embryosac is reckoned with. Too many points however are still left out of consideration. Though improved the system remains artificial.

PALM (1915) who in a more extensive study advocates the same scheme rightly remarks: "Das diese Aufteilung nur eine künstliche sein kann ist ja selbstverständlich." Its lack of phylogenetical value is best shown by reproducing PALM's scheme, in which the different types are called after their first representative known:

	5 Teilungen.	4 Teilungen.	3 Teilungen.	2 Teilungen.
1 megasp.	Normaltypus.	Codiaeumtypus.	Dicraeatypus.	——
2 megasp.	——	Scillatypus.	Cypripedium-typus.	——
3 megasp.	——	Peperomiatypus.	Liliumtypus.	Plumbagella-typus.

Ishikawa (1918) lately published a scheme which from a phylogenetical point of view is certainly to be marked as a distinct progress. He no longer sticks to one or two rather voluntarily chosen moments in the development of the embryosac, but he tries to reckon with the other facts as well. His scheme (Ann. of Bot. 32, p. 305, Fig. XI) not only deals with the number of divisions and with the number of megaspores which join in embryosac-formation, but also pays attention to the origin and to the number of nuclei in the full-grown sac. So it really contains some necessary elements for the building up of a natural system.

When put into practice however its usefulness is rather limited. It meets our present knowledge of embryosacs, but is not planned broadly enough to include further possibilities. It gives an insight in the author's views on homologies, but it does not give a valuable system. It is meant to give much, but it is worked out confusedly. Perhaps that is the reason why all harmony with the sporophytic system is absolutely lacking.

Objections against Coulter's principles.

As the most succesful arguments against the view, the development which of we just finished sketching, the following has been brought forward:

1°. Against the assumption that megaspore-formation cannot be shortened and always must be preceded by two divisions, was moved the fact that the sporogenous tissue has gradually been restricted from an elaborate tissue among Gymnosperms to only one cell in most Angiosperms. There is no reason why this tendency to shorten the gametophyte-generation should have stopped there. On the contrary we might expect this tendency to go on and affect megaspore-formation. It gives a natural explanation of the reduction series as demonstrated in the "row of four", "of three", "of two", "no row at all."

2°. Against the assumption that megaspore-formation should be determined by chromatine-reduction, was moved Murbeck's (1901) discovery of the embryosac-development in Alche-

milla, in which species a row of four is quite normally formed, without any reduction in the number of chromosomes. It seems hardly possible not to homologise this row of four with the ordinary row of four megaspores, especially since one of the four develops to a normal 8-nucleate embryosac.

3°. Against the assumption that cellwall-omission should induce the development of two or more megaspores in the same cell, was moved the fact that no cases are known of four unquestionable megaspores, lying in the same cell and developing all four to form one embryosac of the usual 8-nucleate type. On the contrary it is difficult to see why *four* developing megaspores should arrange their nuclei into *two* groups (a micropylar and a chalazal one), just as in an ordinary monosporical sac and without leaving any trace of the tetrasporical origin.

4°. Against the assumption that the number of nuclei in the full-grown sac should be of no importance at all, was moved the fact that the 8-nucleate sac is of such remarkable frequency, that its appearance cannot be believed to be mere chance. The less so since (according to Coulter) these eight nuclei represent either the greatgranddaughters (in monospor. sacs), or the granddaughters (in bispor. sacs), or the daughters (in tetraspor. sacs) of the megaspore-nuclei.

Ernst's opinion.

Ernst (1908a, 1908b) rejecting Coulter's view and all classification based on the number of divisions points out that:

1°. Two distinct processes can be recognised in the life history of the gametophyte, viz. embryosac-*formation* and embryosac-*development*. "Die Entwicklungsvorgänge im Embryosack scheinen mir unabhängig von seiner Entstehung betrachtet werden zu müssen." "Die fünf Teilungen gehören ja ganz verschiedenen Entwicklungsvorgängen an." "*Die beiden ersten repräsentieren die letzten Teilungen in einem Makrosporangium*... und gehören dem Vorgang der Sporen*bildung* an." "Die drei anderen Teilungen dagegen erfolgen im Verlaufe der Sporen*keimung*" (1908b, S. 26).

2°. The process of embryosac-*formation* may be affected by reduction. Instead of four, only three or two megaspores are formed, or even the embryosac-mothercell itself functions as megaspore. Thus chromatine-reduction, ordinarily occurring during embryosac-formation, necessarily is transferred to a later stage. "Bei teilweiser Unterdrückung der Tetradenteilung wird der zweite Teilungsschritt der Reduktionsteilung in die keimende Spore verlegt, und bei vollständig ausbleibender Tetradenteilung finden beide der zur *Reduktion notwendigen Teilungen innerhalb der keimenden Makrospore statt.*" (S. 27).

3°. The process of embryosac-*development* is wholly independent of that of embryosac-formation. Among Liliaceae e. g. all types of megaspore-formation are found, from the normal tetrad down to total suppression. Always however the functioning megaspore — by three divisions — reaches the 8-nucleate stage.

4°. The process of embryosac-*development* is determined by the number of divisions, by the arrangement of the nuclei, by vacuolation and by cell-formation.

On these grounds Ernst, when reviewing the literature, concludes that two types of embryosacs can be recognised, viz. the ordinary eight-nucleate one and a sixteen-nucleate type, "als ältere oder doch als selbstständige Form des Embryosackes der Angiospermen" (S. 29). This more primitive type is distinguished by the divisions numbering four instead of three, by the absence (at least at first) of a central vacuole, and by the lack of bipolarity. The 16-nucleate sac thus shows itself to be of a primitive character as to embryosac-development, and of a reduced nature as to embryosac-formation, there being no row at all, the embryosac-mothercell itself functioning as embryosac.

This marked distinction between embryosac-formation and development is a real advantage on Coulter's system. It is a first step on the way of treating the various processes of the female gametophyte separately. So far it opens the prospect of getting a natural system. Laying all the stress however on

the total number of nuclei in the mature sac, without paying any attention to their origin, makes Ernst's system almost as artificial as Coulter's. For there are still many more factors, which show an independent line of development in the life history of the gametophyte. That is why a lot of abnormal embryosacs, discovered since Ernst published his system, could not be placed in his scheme.

To complete this review of systematical and phylogenetical studies on Angiosperm embryosacs, we have still got to mention the publications of Campbell, of Jacobsson-Stiasny and of Schürhoff.

Campbell (1899, 1900, 1902, 1903, 1905, 1909, 1910, 1911, 1912) in a series of articles tried to propagate the idea that "the embryosacs with an increased number of nuclei are older types" (1911). It is only the *number* of nuclei that counts with him; their *origin* is not thought worth much attention. According to him there is a gradual passing on from the multicellular Gymnosperm type to the ordinary 8-nucleate Angiosperm sac: Pandanus with its 32—64 antipodal nuclei, is "really primitive", and the 16-nucleate sacs of Peperomia and Gunnera form the transition to the normal type.

Emma Jacobsson Stiasny (1916) rightly states "im Gegensatz zu Ernst, dasz die Anzahl der Kerne des reifen Embryosackes, im Gegensatz zu Coulter, dasz die Anzahl der Teilungen allein noch nicht zur Charakterisierung der Stellung genügen kann." Her "kausalmechanische Darstellung" however is at least as one-sided as any of the older systems. The whole study is based on the idea of "Ernährungsverhältnisse" being the only possible cause of any atypical number of nuclei in the embryosac. Far too much importance is attributed to the number of nuclei in the full-grown sac, and scarcely any attention is paid to their origin. Without further investigation the 16-nucleate sacs are accepted to represent a type of their own.

Schürhoff (1919) is only mentioned here because his publication is of so recent a date. It will do to state:
1°. That his system is *based* on the absolutely false assumption

that one synergid should be a sister to the egg and the other to the upper polar nucleus! All students however agree on both synergids being sisters, but the author, appearing to throw aside his usual powers of self-criticism, moves as conclusive proof in favour of his view: "Da nach meiner Erklärung die eine Synergide eine Schwesterzelle des oberen Polkerns ist...."

2°. That lots of his further arguments are taken from publications over fifteen years old and which have never since been confirmed.

3°. That his arguments are sometimes misleading as he cites from preliminary notes which have been rectified later on (e. g. his quotations from CAMPBELL on Pandanus, 1909, and from STEPHENS on Penaeaceae, 1908, revised by these authors resp. in 1911 and in 1909).

III. OUTLINES FOR A NATURAL SYSTEM.

Our review of literature has led us to discern two sets of systems, the one based on the number of divisions from embryosac-mothercell up to the egg, and the other on the number of nuclei in the full-grown sac. All of these schemes however were artificial and none of them succeeded in giving an insight in origin and phylogeny of the Angiosperm sac.

Before beginning our attempt at a natural system of the gametophyte it will be good to remember sporophytical conditions which show that each part of the plant follows its own line of development. That is why descent never can be stated with absolute certainty, phylogeny always depending on a *complex* of data. This, of course, must be applied to the study of the gametophyte as well. A system based on the number of nuclei in the full-grown sac (ERNST) or on the number of divisions from mothercell to egg (COULTER) can hardly be considered of greater value for phylogenetical purposes than a sporophytical system based on the number of anthers only. If we want to detect the relations between the eight-nucleate sac and the abnormal ones, we must make clear first how many individual morphological characters can be recognised in the female game-

tophyte. Usually this gametophyte is considered a morphological unit. One can hardly deny, however, that it is of a complex nature and that its morphology has to reckon with the following processes:

Chromatine-reduction.

Megaspore-formation.

Polarisation.

Development of a micropylar group of nuclei.

Development of a chalazal group of nuclei.

If we want to obtain results of phylogenetical value we have got to study the morphology of each of these processes in detail. COULTER and his school identified chromatine-reduction and megaspore-formation, and likewise ERNST mixed up two lines of development when basing his system on the total number of nuclei, without paying any attention to their origin. Of course it is quite possible that there exist connections like those suggested by COULTER and ERNST, but there is no good in presupposing them. If they exist they will come to light even when treating the processes separately.

We will first discuss chromatine-reduction and polarisation. These two seem to be processes of great constancy and are very seldom, if ever, affected by deviation from the normal.

Reduction division.

For years this process has been one of the main objects of cytological research. It is not necessary to give a description in detail of the phenomenon, the more since it is apparently without any phylogenetical value. With the few exceptions, presented by apogamous plants, reduction division always occurs immediately after the formation of the embryosac-mothercell. The process is not affected by deviations in megaspore formation, and seems to be of the utmost constancy. It goes on in embryosac-mothercells, resulting in four megaspores, as well as in embryosacs derived from the mothercell without any megaspore formation.

Polarisation.

It is really astonishing that a phenomenon so obvious as

polarisation is, has never been subjected to a special study. Its origin, its inducing factors, its function, etc. are entirely unknown, which is the more remarkable since everyone who has ever studied the development of an embryosac must be familiar with the process. It goes beyond the scheme of this paper to expatiate on questions connected with its meaning. From our point of view it will do to state that the process goes on in all embryosacs in absolutely the same way. The formation of the well known large central vacuole is the most prominent phenomenon that accompanies polarisation and therefore worth our special attention. In the normal eight-nucleate sac, sprung from a "row of four" no trace of vacuolation[1]) is to be seen during megaspore-formation. Protoplasm remains a homogenous mass until the development of the embryosac (megaspore) begins. Then vacuolation commences as a group of small vacuoles which soon results in the usual large central vacuole. This process passes with such rapidity that even the two-nucleate stage could never be found without the typical large vacuole, which separates both nuclei, indicating them as primary micropylar and primary chalazal nucleus. This normal course of vacuolation is so common that most authors do not even mention it. As a matter of fact polarisation and vacuolation of the embryosac are mostly left out of discussion, only few authors indicating them. Sometimes even their figures are but outlined, all information on the subject thus lacking. Modilewski (1909) seems to have felt its significance when saying: "Man musz aber womöglich nicht nur dieses Merkmal (die Zahl der Kerne) sondern auch die Höhe der Symmetrie und die Polarität des Embryosacks in Betracht ziehen. Dan wird man vielleicht imstande sein, einige Anhaltspunkte zu gewinnen." No attention at all, however, is paid to these words. A thorough study of the publications (including their figures!) on abnormal embryosacs will show the correctness of Modilewski's remark and the fundamental significance

1) It is not the intention to give any opinion on the origin and formation of the vacuoles. Here and in the following the word "vacuolation" is only used for indicating the appearence of the central vacuole.

of the process of vacuolation for homologising the different stadia in the development of atypical sacs. The process just described and well-known to all students of morphology, gives cause to call the attention to the following points:

1°. *Polarisation (vacuolation) is a function of the embryosac (developing megaspore). It does not accompany the megaspore-formation, but its development. It commences as soon as megaspore development begins.*

This remarkably constant character, viz. vacuolation just preceding the first division of the functioning megaspore, furnishes us with a new characteristic by which megaspores may be recognised, even when the "row of four" is not formed. As long as plasm remains homogenous, megaspore *formation* is still going on. As soon as vacuolation commences, megaspore *development* has begun. So the nuclei just preceding vacuolation are to be considered as megaspore nuclei.

I am well aware that this proposed use of vacuolation as means of recognising megaspores is nothing more than a working hypothesis. Its progress in the normal sac is no reason in itselt to assume that vacuolation always and under all circumstances should be bound to the early stages of spore-germination. Therefore I will move some arguments in favour of the hypothesis.

Firstly in literature no case is met with in which vacuolation did not commence just after megaspore-formation.

Secondly the hypothesis is confirmed by all well-established and undoubtable cases of megaspore-formation under abnormal conditions (which fully justifies the application of the idea to those cases in which homologising meets with difficulties). For instance: Smith (1911) describes the embryosac-mothercell-nucleus of Clintonia giving rise to a row of four nuclei, not separated by cell walls. Three of these soon desintegrate, only the upper one developing. Nobody will dispute the megaspore-character of these four nuclei. Though all four megaspores are lying in the same cell, *plasm remains homogenous* up to the first division of the developing nucleus. According to Johnson (1914) the walls between the megaspores in Peperomia hispidula are very delicate and soon disappear, leaving four nuclei in a *continuous*

mass of cytoplasm. During the preparation for the next division the large central vacuole is rapidly formed. In Peperomia Sintensii (Brown, 1908) an evanescent wall appears in the first division only; in other Peperomia's (Campbell, 1899; Johnson, 1900) cell wall formation is wholly omitted, but in all these cases cytoplasm remains homogenous up to the third division (= first division of the megaspores).

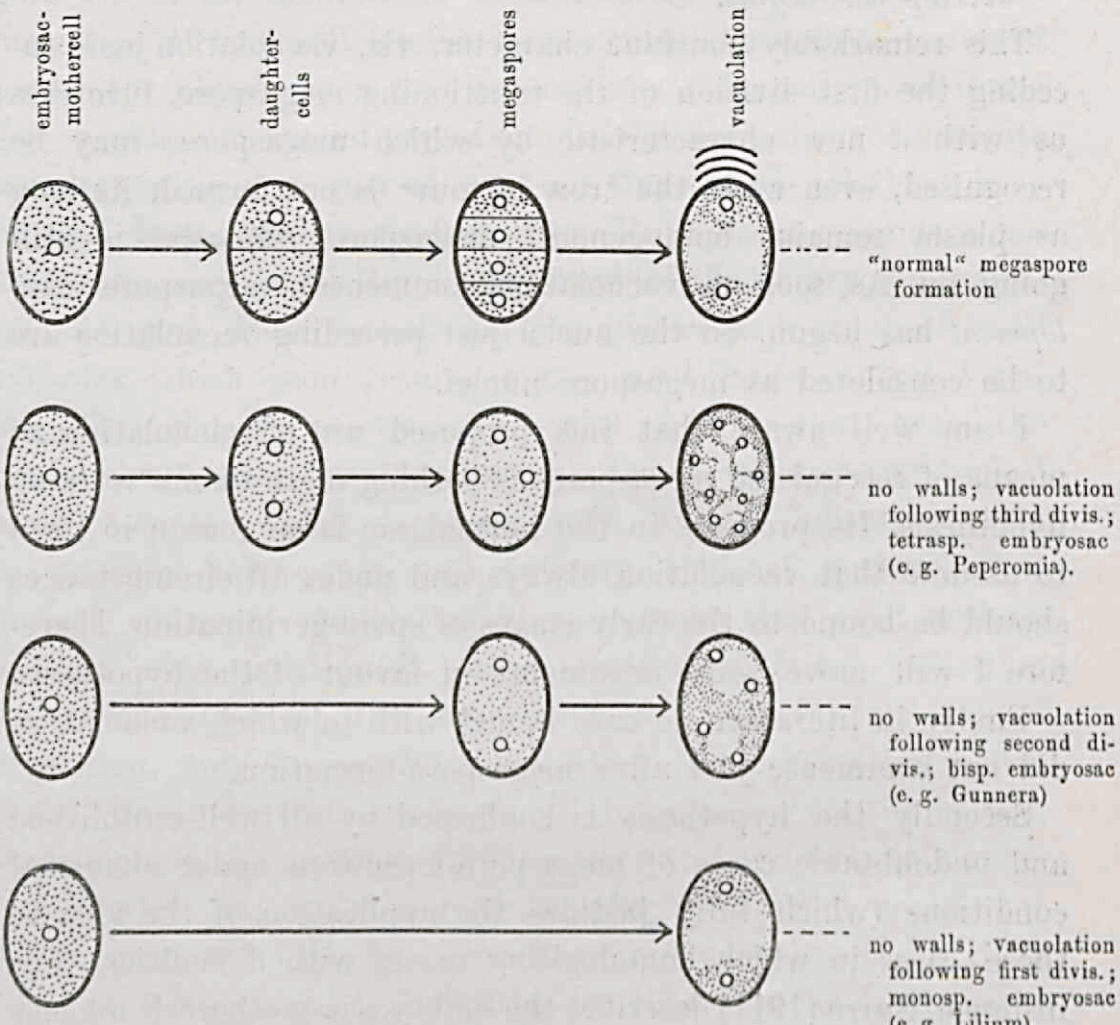

Fig. 2. Polarisation (vacuolation) as means of recognizing megaspores.

Thirdly we get results from the application of the hypothesis: Up till now vacuolation was entirely left out of consideration by all authors, and embryosacs were considered of identical development, whether showing vacuolation after the first or second or third division. So for instance any reasonable explanation of conditions in Gunnera with its seven fusing nuclei and in Peperomia with its eight fusing nuclei, was absolutely lacking.

When marking vacuolation, differences will be noticed in the early stages of embryosac-formation in e. g. Lilium, Gunnera and Peperomia, and they will be recognised as being of resp. mono-, bi- and tetrasporical character (Fig. 2). Likewise the peculiar number of eight fusing nuclei in Peperomia (Fig. 9) and of seven in Gunnera (Fig. 11) loses its mystery. It is not necessary to supply further examples here; the hypothesis' working capacities will become more and more clear in the following pages.

2°. *In the two-nucleate stage of a normal embryosac the nuclei are always separated by the large central vacuole (the embryosac is polarised).*

The significance of this central vacuole for homologising purposes is plain. It provides us with means to distinguish the nuclei of the micropylar end from those of the chalazal end. There is no difficulty whatsoever in distinguishing the primary micropylar nucleus and the primary chalazal one. Only few authors however have realized the significance of this polarisation. As we will point to it often later on, one instance will do for the moment to illustrate its extraordinary value: In Onagraceae all four nuclei of the mature sac are found at the micropylar end. The likewise four-nucleate Plumbago sac shows two nuclei at each end of the central vacuole. In the first case all nuclei are of micropylar origin, in the second one two are micropylar and two chalazal. Though both sacs are four-nucleate it is undoubtedly a mistake to homologise these two. From the very beginning of their development they are plainly different.

Megaspore-formation.

Normally a "row of four" is formed, one of which becomes the functioning megaspore, Whether the arrangement of these cells is ⋮ or ⁘ or ∴, and whether the inner one or any of the other three becomes the functioning megaspore [1]), and even

1) Discussion and complete literature by PALM (1915, p. 110).

whether one or more of these megaspore-cells develop [1]), are questions most probably of systematical interest as well, but lying beyond the scheme of this study. It is the *number* of megaspores formed, that interests us now, and whether it is possible that two or more megaspores enter in embryosac-formation, thus affecting the number of nuclei in the mature sac. When formulating these two points more exactly, it turns out that we have got to study the following lines of deviation from the normal type:

1°. *The possibilities of a reduction in the number of megaspores.*

It is known and needs no further commentary that the usual number of megaspores is four. Normally three of these desintegrate as soon as embryosac-development begins. Theoretically however we might as well expect a partial or total suppression of these non-functioning nuclei. The development of a reduction in this direction is fully worked out and represented in fig. 3. The (normal) "row of four" is figured by A. B shows the "row of three", C the "row of two" and in D "the embryosac-mothercell itself is seen functioning as an embryosac." In agreement with this gradual suppression, respectively three, two, one or none megaspores are seen desintegrating.

It must be emphasized here again that this reduction series rejects all presupposed connections between megaspore-formation and chromatine-reduction. When there is a row of four or a row of three (fig. 3 A, B) chromatine-reduction is finished before embryosac development begins and so coincides with megaspore-formation. When however there is only a row of two (fig. 3 C) the second reduction division — and when there is no row at all (fig. 3 D) both divisions — are shifted into the germinating megaspore.

We have already moved some arguments in favour of our treating these two processns separately (p. 15, 16 and p. 19). Moreover the correctness of this conception will be confirmed later on, this idea of a reduction series being in full accordance with the vacuolation process.

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1) Complete literature by PALM (1915, p. 110—144).

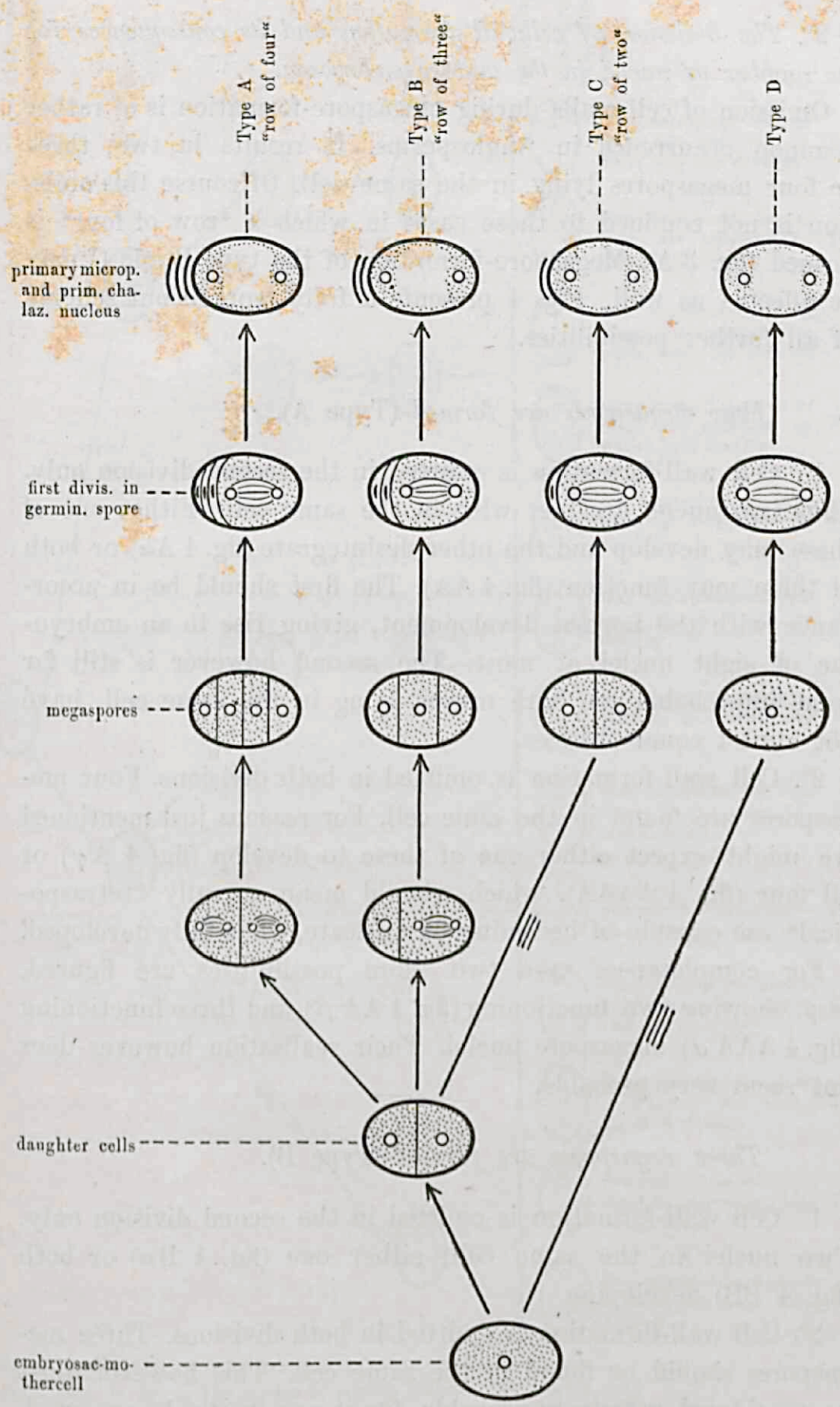


Fig. 3. Megaspore formation.

2°. *The omission of cellwall formation and its consequences for the number of nuclei in the mature embryosac.*

Omission of cell walls during megaspore-formation is of rather common occurrence in Angiosperms. It results in two, three or four megaspores lying in the same cell. Of course this omission is not confined to those cases in which a "row of four" is formed (fig. 3 A). Megaspore-formation of the type B and C may be affected as well. Fig. 4 presents a fully worked out scheme of all further possibilities.

Four megaspores are formed (Type A).

1°. Cell wall-formation is omitted in the second division only. Thus two nuclei are met with in the same cell. Either one of these may develop and the other desintegrate (fig. 4 Aα) or both of them may function (fig. 4 AA). The first should be in accordance with the normal development, giving rise to an embryosac of eight nuclei at most. The second however is still far from improbable for both nuclei, lying in the same cell, have got almost equal chances.

2°. Cell wall-formation is omitted in both divisions. Four megaspores are found in the same cell. For reasons just mentioned we might expect either one of these to develop (fig. 4 Aγ) or all four (fig. 4 AAAA), which whould mean a really "tetrasporical" sac capable of becoming 32-nucleate, when fully developed.

For completeness' sake two more possibilities are figured, resp. showing two functioning (fig. 4 AA β) and three functioning (fig. 4 AAA α) megaspore nuclei. Their realisation however does not seem very probable.

Three megaspores are formed (Type B).

1°. Cell wall-formation is omitted in the second division only. Two nuclei in the same cell, either one (fig. 4 Bα) or both (fig. 4 BB) developing.

2°. Cell wall-formation is omitted in both divisions. Three megaspores should be found in the same cell. This however must be considered utterly improbable, for it can hardly be expected,

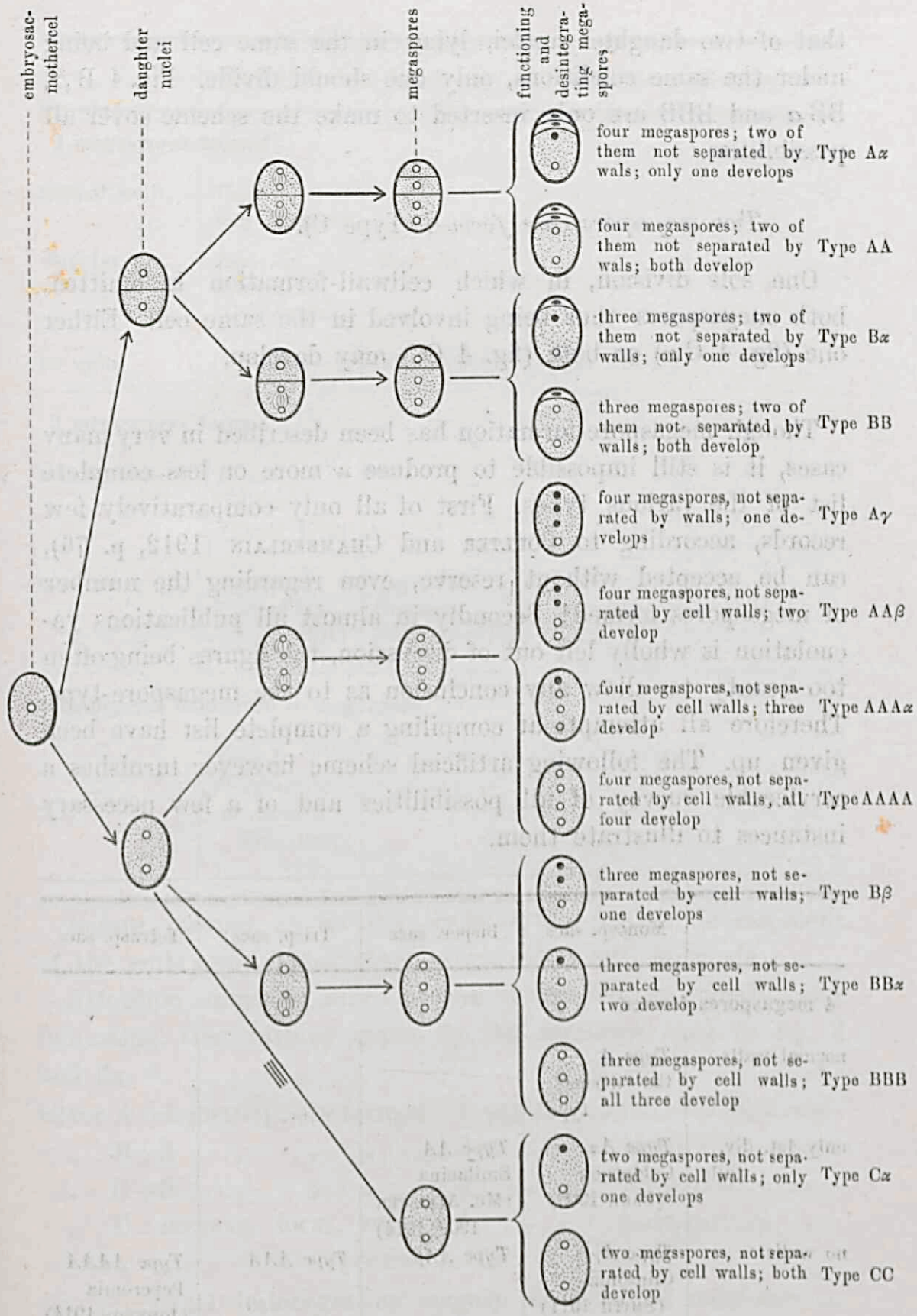


Fig. 4. *Origin of bi-, (tri-) and tetrasporical sacs.* Letters corresponding to those used in fig. 3. The number of capitals indicates the number of spores entering in embryosac formation. The greek letter indicates how many desorganizing megaspores are met with within te same sac.

that of two daughter nuclei, lying in the same cell and being under the same conditions, only one should divide. Fig. 4 B β, BB α and BBB are only inserted to make the scheme cover all possibilities.

Two megaspores are formed (Type C).

One sole division, in which cellwall-formation is omitted, both megaspores thus being involved in the same cell. Either one (fig. 4 C α) or both (fig. 4 CC) may develop.

Though megaspore-formation has been described in very many cases, it is still impossible to produce a more or less complete list of the various types. First of all only comparatively few records, according to COULTER and CHAMBERLAIN (1912, p. 76), can be accepted without reserve, even regarding the number of megaspores formed! Secondly in almost all publications vacuolation is wholly left out of discussion, the figures being often too scanty to allow any conclusion as to the megaspore-type. Therefore all attempts at compiling a complete list have been given up. The following artificial scheme however furnishes a serviceable survey of all possibilities and of a few necessary instances to illustrate them.

	Monosp. sacs	Bispor. sacs	Trisp. sacs	Tetrasp. sacs
4 megaspores formed				
normal walls	*Type A* the "normal type"			
only 1st. div. walls	*Type Aα* Calopogon (PACE 1909)	*Type AA* Smilacina (MC. ALLISTER 1909, 1914)		
no walls	*Type Aγ* Clintonia (SMITH 1911) Avena (CANNON 1900)	*Type AAβ*	*Type AAA*	*Type AAAA* Peperomia (JOHNSON 1914) Penaeaceae (STEPHENS 1909)

	Monosp. sacs	Bispor. sacs	Trisp. sacs	Tetrasp. sacs
3 megaspores formed				
normal walls	*Type B* very common			
only 1st. div. walls	*Type Bα* Gyrostachys (PACE 1914)	*Type BB*		
no walls	(*Type Bβ*)	(*Type BBα*)	(*Type BBB*)	
2 megaspores formed				
normal walls	*Type C* Trillium (ERNST 1902)			
no walls	*Type Cα* Piper (PALM 1915)	*Type CC* Gunnera (SAMUELS 1912)		
Embryosac mothercell = megaspore				
	Type D Plumbagella (DAHLGREN 1915, 1916)			

Before passing on to the study of the further development of the embryosac, three observations must still be made.

Attention must be called to the system which underlies our indicating the various types by the formules used in fig. 3 and fig. 4.

letter A : 4 megasp. are formed
„ B : 3 „ „ „
„ C : 2 „ „ „
„ D : megasp. form. suppressed

1 capital : monosporical sac
2 „ s : bisporical „
3 „ s : trisporical „
4 „ s : tetrasporical „

α : 1 desintegrating megasp. nucl. in the same sac
β : 2 „ „ „ „ „ „ „
γ : 3 „ „ „ „ „ „ „

Further it must be emphasized that our terms: mono-, bi- and tetrasporical are by no means identical with those of COULTER's. Deduction has lead us to distinguish;

nine types of monosp. sacs, viz. A, Aα, Aγ, B, Bα, (Bβ), C, Cα, and D.
five types of bispor. sacs, viz. AA, (AAβ), BB, (BBα), and CC.
(two types of trispor. sacs, viz. AAAα, and BBB).
one type of a tetrasporic. sac, viz. AAAA.

COULTER however, identifying megaspore-formation and chromatine-reduction, discerns three types only:

monosporical sacs (including our types A and B)
bisporical sacs (including our types C, AA and BB)
tetrasporical sacs (including our types D, AAAA and CC).

This distinction is not a theoretical question of nomenclature, but based on a real difference. Our 17 types are no fancies but plainly distinguishable forms. For instance Lilium, Peperomia and Gunnera are all three considered from COULTER's point of view, to be of a tetrasporical nature and of the same megaspore-formation. In fact however the early stages of development are *not* the same, vacuolation following the first division of the embryosac-mothercell in Lilium, the second division in Gunnera and the third division in Peperomia. Lilium therefore must be classified as belonging to type D, Gunnera as to type CC and Peperomia as to type AAAA (fig. 2, p. 22).

Thirdly it is good to point out again, that actually *two* lines of development are joined in fig. 4, viz. the omission of cell-wall-formation and the germinating of two or more megaspores. Of these two the first line is fully worked out; the other one however only inasfar as it coincides with the first one and thus influences the number of nuclei in the developing embryosac. As already stated above, we have left out of discussion the very many cases in which two or more normally formed megaspores (separated by cell walls) are seen functioning.

Development of the micropylar group of nuclei.

Normally the primary micropylar nucleus by two successive divisions gives rise to a group of four nuclei. Spindles in the second division are almost always showing "|" shape. The upper

sister nuclei are the synergids, the other two being the egg and the upper polar nucleus.

Often the development of this group is affected by a reduction in the number of nuclei. Theoretically an increase should be possible as well, but instances of a regular occurence of more than four nuclei are not known.

The reduction series is worked out in figure 5, the number

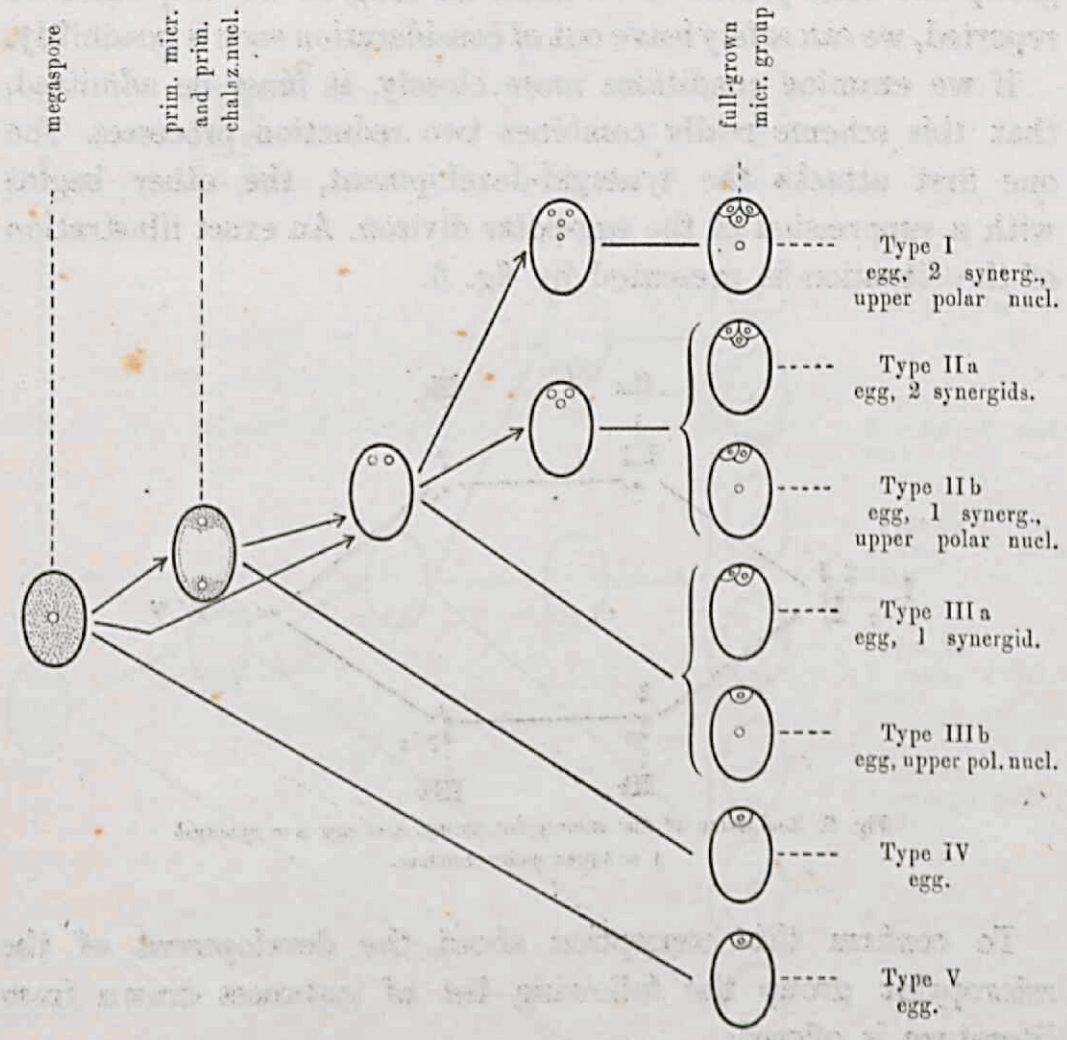


Fig. 5. Reduction of the micropylar group.

of nuclei ranging from four to one. A further reduction should not be possible, for the character of the gametophyte resists against total suppression and requires at least one nucleus: the egg. When four nuclei are present, one is the egg, one the upper polar nucleus and two are synergids (fig. 5 I). Three nuclei may be either the egg and two synergids (fig. 5

IIa) or the egg, the upper polar and one synergid (fig. 5 IIb). Two nuclei are egg and synergid (fig. 5 IIIa) or egg and polar (fig. 5 IIIb). One nucleus necessarily must be the egg, being either the undivided primary micropylar nucleus (fig. 5 IV) or possibly even the undivided megaspore itself (fig. 5 V). A suppression of the egg does not seem very probable. The case of Dasylirion in which there should be no egg in the micropylar group has been proved to be false. As long as no new cases are reported, we can safely leave out of consideration such a possibility.

If we examine conditions more closely, it must be admitted, that this scheme really combines two reduction processes. The one first attacks the synergid-development, the other begins with a suppression of the egg-polar divison. An exact illustration of the situation is presented by fig. 6.

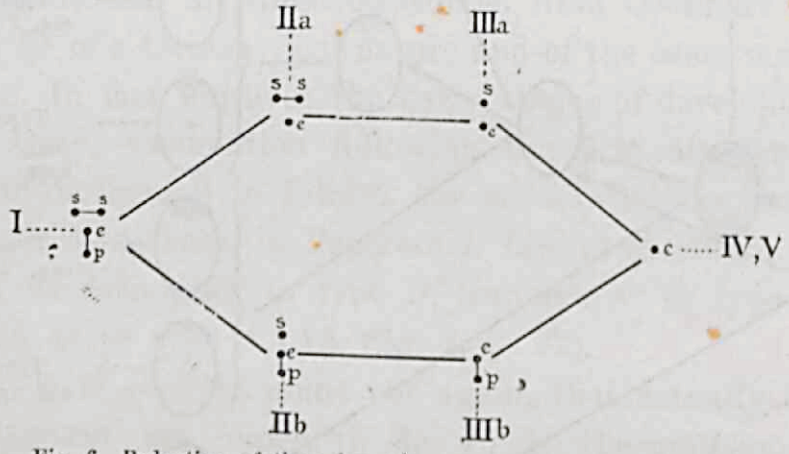


Fig. 6. Reduction of the micropylar group, e = egg s = synergid p = upper polar nucleus.

To confirm this conception about the development of the micropylar group the following list of instances drawn from literature is offered.

Type I : the "normal development".
Type II*a* : Aglaonema (Campbell, 1912), Garcinia (Treub, 1911), Moringa (Rutgers, 1922), Cypripedium (Pace, 1907), Gastrodia (Kusano, 1915).
Type II*b* : (Juglans regia (Karsten, 1902) ?)
Type III*a* : Peperomia (Johnson, 1900, 1907, 1914, Brown, 1908) (Dicraea elongata (Magnus, 1913) ?)

Type III*b* : Plumbagella (DAHLGREN, 1915).
Type IV : Plumbago (DAHLGREN, 1915).
Type V : . . . . . .

Development of the chalazal group of nuclei.

The primary chalazal nucleus also, normally develops into a group of four nuclei: the lower polar nucleus and three antipodals.

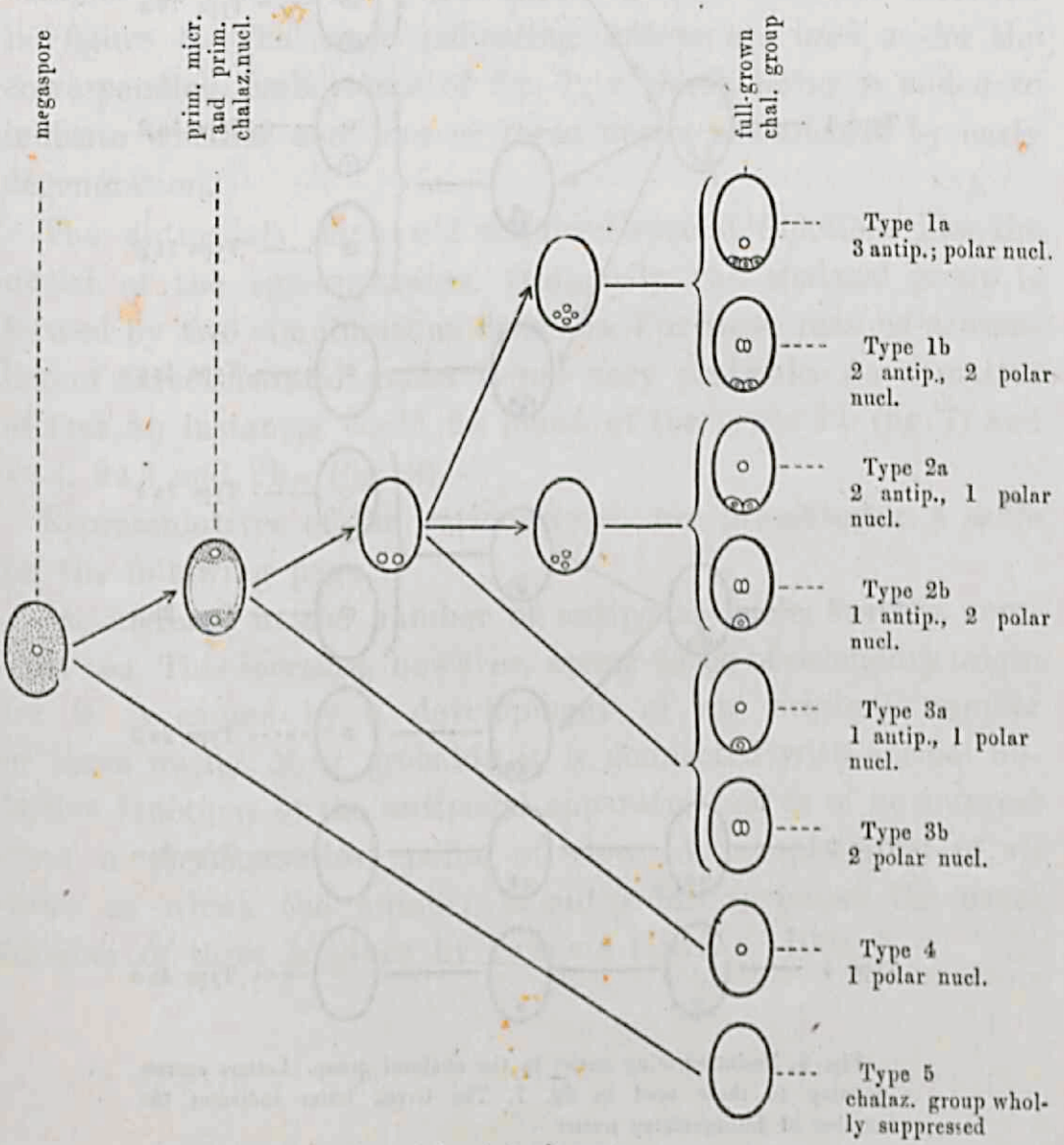


Fig. 7. Reduction of the chalazal group.

The number of nuclei however may be either more or less. Many instances both of suppression and of secondary increase are known.

Figure 7 illustrates the theoretical reduction series. The scheme covers all possibilities from the normal number of four

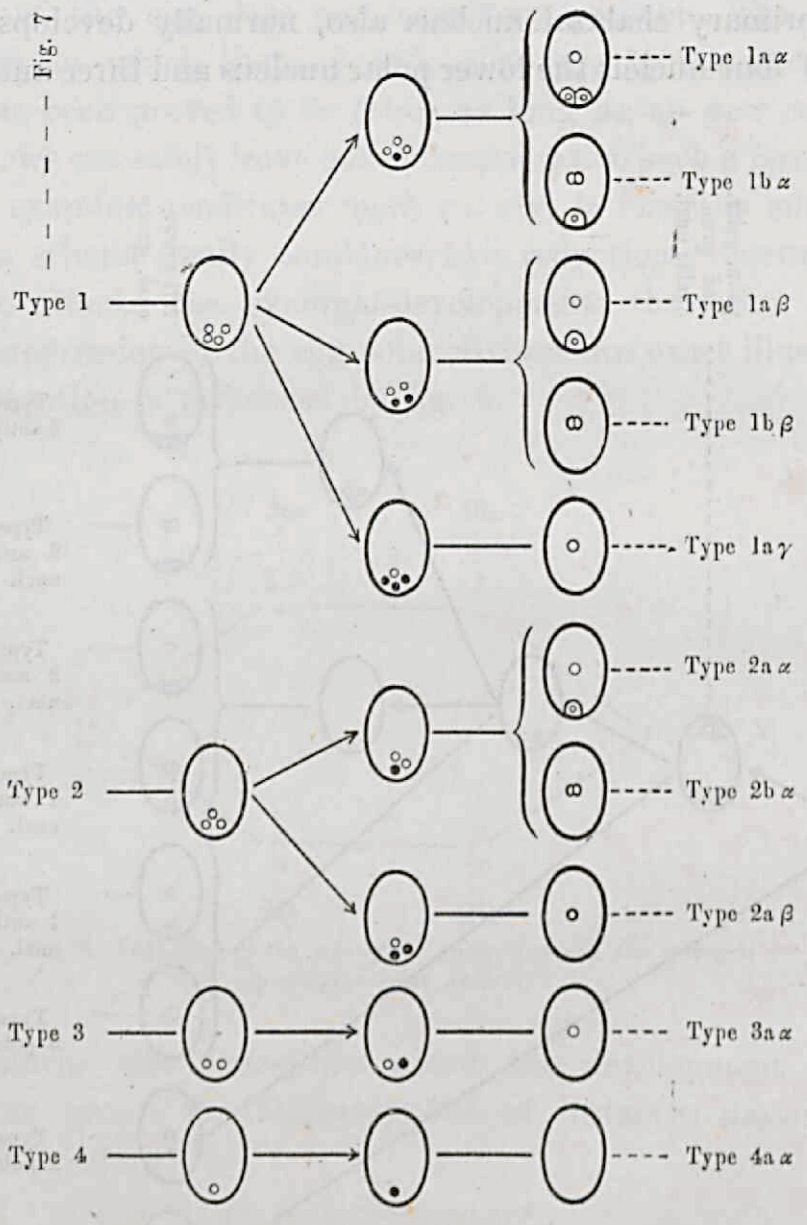


Fig. 8. Desintregrating nuclei in the chalazal group. Letters corresponding to those used in fig. 7. The Greek letter indicates the number of desorganizing nuclei.

nuclei down to total suppression of the entire group. Sometimes, when the upper polar nucleus is suppressed (fig. 5,

IIa, IIIa, IV, V) two of the chalazal nuclei are seen functioning as polars and even fusing.

Besides the reduction just mentioned there is still another way by which the number might decrease. Antipodals very seldom survive the fertilization stage. Often however they begin desintegrating long before the sac has reached its full-grown stage. This of course is to be considered as an anticipation without much interest from a morphological point of view. For completeness' sake all possibilities on this line are reviewed in figure 8. The same indicating letters are used as for the corresponding embryosacs of fig. 7; a Greek letter is added to indicate whether one, two or three nuclei are affected by early degeneration.

The antipodals have not acquired special functions like the nuclei of the egg-apparatus. Ordinarily the chalazal group is formed by two simultaneous divisions. For these reasons a number of three chalazal nuclei is not very probable. As a matter of fact no instances could be found of the types 2b (fig. 7) and 2aα, 2aβ and 2bα (fig. 8).

Representatives of the various types are presented in a table on the following page.

An increase in the number of antipodal nuclei is very common too. This increase, however, seems to be of secondary origin for it is caused by a development of an original number of three nuclei. Most probably it is connected with special nutritive functions of the antipodal apparatus and is of no interest from a phylogenetical point of view. A complete list of all cases in which the number of antipodals surpasses the usual number of three is given by Samuels (1912 p. 100).

group of 4 chalazal nuclei	Type 1a: the "normal development" Type 1b:	Type 1aα–1aβ–1aγ: quite common Type 1bα–1bβ: . . . . .
group of 3 chalazal nuclei	Type 2a: Pedilanthus (ARNOLDI 1912) Type 2b:	Type 2aα–2aβ: . . . . . . Type 2bα: . . . . .
group of 2 chalazal nuclei	Type 3a: Dicraea (MAGNUS 1913)? Limnocharis (NITZSCHKE 1914) Gyrostachys (PACE 1914) Epipactis (BROWN & SHARP 1911) Type 3b: Peperomia (CAMPBELL 1899, JOHNSON 1900, 1907, 1914, BROWN 1908). Garcinia (TREUB 1911) Moringa (RUTGERS 1922) Broughtonia and other Orchidac (SHARP 1912) Cypripedium (PACE 1907) Gastrodia (KUSANO 1915) Aglaonema (CAMPBELL 1903, 1912)	Type 3aα: Codiaeum (ARNOLDI 1912) Plumbagella (DAHLGREN 1915) Gyrostachys (PACE 1914)
1 chalazal nucleus only	Type 4: Lawia (MAGNUS 1913)	Type 4aα: Helosis (CHODAT & BERNARD 1900) Ceramanthus (ARNOLDI 1912) Podostemac. (WENT 1909, 1910, 1912)
chalazal group wholly suppressed	Type 5: Euphorbiac (MODILEWSKI 1909a, 1910, 1911, ARNOLDI 1912) Podostemac. (MAGNUS 1913) Penaeac. (STEPHENS 1909) Onagrac. (GEERTS 1909, MODILEWSKI 1909b, WERNER 1914, RENNER 1914, ISHIKAWA 1918).	

IV. SYSTEMATICAL SURVEY OF ATYPICAL EMBRYOSACS.

As most authors have not paid much attention to the distribution of the protoplasm and to the arrangement of the nuclei during the early stages of embryosac development, this review necessarily must be a critical one, including not only the interpretation of the figures as given by the authors, but a discussion of the figures themselves as well.

The common desintegration of an otherwise normal group of antipodals (fig. 8, 1aα, 1aβ, 1aγ) as well as the secondary increase of the number of antipodals, are not included. For the rest the criticism reckons with all embryosacs, known to have more or less than the ordinary number of eight nuclei, and it thus covers the whole range of possibilities represented in fig. 4—8.

DICOTYLEDONES — *Choripetalae.*

Juglandaceae	Juglans regia	7-nucleate?	Karsten	1902	D-IIb-1a?
Balanophoraceae	Helosis guyanensis	4-nucleate	Chodat et Bernard	1900	D-I-4α
Piperaceae	Peperomia pellucida	16-nucleate	Campbell	1899	AAAA-IIIa-3b
			Johnson	1900	
	Peperomia hispidula	16-nucleate	Johnson	1907, 1914	AAAA-IIIa-3b
	Peperomia Sintensii, Peperomia arifolia, Peperomia Ottomania	16-nucleate	Brown	1908	AAAA-IIIa-3b
	Peperomia resediflora, Peperomia blanda, Peperomia marmorata, Peperomia magnoliifolia	16-nucleate	Häuser	1916	AAAA-IIIa-3b
	Piper subpeltatum	occasionally 5-nucleate	Palm	1915	Cα-IIIa-3b
Euphorbiaceae	Ceramanthus	4-nucleate	Arnoldi	1912	A-I-4α
	Codiaeum	4-nucleate	Arnoldi	1912	A-I-3aα

Euphorbiaceae	Pedilanthus	5-, occas. 7-nucleate	Arnoldi	1912	A-I-4 A-I-2a
	Euphorbia procera	16-nucleate	Modilewski Modilewski	1909 1910	AAAA-I-5
	Euphorbia virgata	16-nucleate	Dessiatoff Modilewski	1911 1911	AAAA-I-5
	Euphorbia palustris	16-nucleate	Modilewski	1911	AAAA-I-5
	Acalypha	16-nucleate	Arnoldi	1912	AAAA-I-5
Guttiferae	Garcinia Kydia Garcinia Treubii	5-nucleate	Treub	1911	A-IIa-3b
Moringaceae	Moringa oleifera	5-nucleate	Rutgers	1922	A-IIa-3b
Podostemaceae	Oenone Imthurni Oenone guyanensis Oenone Richardiana Oenone Treslingiana Oenone Versteegiana Oenone marowynensis Apinagia divertens Apinagia Goejei Apinagia perpusilla Lophogyne capillacea Mourera fluviatilis Tristicha hypnoides	4-nucleate	Went	1909 1910	C-I-4α
	Rhyncholacis macrocarpa Oenone Hulkiana Cladopus Nymanni	4-nucleate	Went	1912	C-I-4α
	Podostemon subulatum Hydrobium olivaceum	5-nucleate	Magnus	1913	C-I-4
	Farmeria metzgerioides Lawia zeylanica	4-nucleate	Magnus	1913	C-I-5
	Dicraea elongata	4-nucleate	Magnus	1913	C-IIIa-3a
Penaeaceae	Sarcocolla squamosa Sarcocolla fucata Sarcocolla formosa Penaea mucronata Penaea ovata Brachysiphon imbricatum	16-nucleate	Stephens	1908 1909	AAAA-I-5
Onagraceae	Oenothera Lamarckiana	4-nucleate	Geerts Werner	1909 1914	A-I-5
	Oenothera biennis	4-nucleate	Modilewski Werner Renner	1909 1914 1914	
	Circaea lutetiana	4-nucleate	Modilewski Werner	1909 1914	
	Epilobium Dodonaei	4-nucleate	Modilewski	1909	
	Epilobium angustifolium	4-nucleate	Modilewski Werner Ishikawa	1909 1914 1918	
	Oenothera rhizocarpa	4-nucleate	Werner	1914	

Onagraceae	Oenothera tetraptera	4-nucleate	Werner	1914	A-I-5
	Oenothera coccinea				
	Fuchsia				
	Clarkia				
	Oenothera nutans	4-nucleate	Ishikawa	1918	
	Oenothera pycnocarpa				
	Gaura Lindheimeri				
	Gaura parviflora				
	Godetia spec.				
	Jussieua repens				
	Ludwigia prostrata				
	Circaea quadrisulcata				
Hallorhagidaceae	Gunnera Hamiltonii	16-nucleate	Schnegg	1902	CC-I-1a
			Ernst	1908	
	Gunnera chilensis	16-nucleate	Modilewski	1908	
	Gunnera macrophylla	16-nucleate	Ernst	1908	
			Samuels	1912	

DICOTYLEDONES — *Sympetalae.*

Plumbaginaceae	Plumbago zeelandica	4-nucleate	Dahlgren	1915	D-IIIb-3a
	Plumbago capensis	4-nucleate		1916	
	Plumbago pulchella	4-nucleate			
	Plumbagella micrantha	3-nucleate	Dahlgren	1915 1916	D-IIIb-3aα
	Ceratostigma plumbaginoides.	4-, occas. 3-nucleate	Dahlgren	1916	D-IIIa-3a (3aα)
Compositae	Pyrethrum parthenifolium var. aureum	16-nucleate	Palm	1914	CC-I-1a
			Palm	1915	

MONOCOTYLEDONES.

Butomaceae	Limnocharis emarginata	5-nucleate	Hall	1902	incorrect
		8/6-nucleate	Nitzschke	1914	A-I-3a (1a)
Liliaceae	Clintonia borealis	4-nucleate	Smith	1911	A-I-5
Orchidaceae	Cypripedium spectabile	4-nucleate	Pace	1907	C-IIa-3b
	Cypripedium parviflorum				
	Cypripedium pubescens				
	Cypripedium candidum				
	Epipactis pubescens	occasionally 6-nucleate	Brown and Sharp	1911	B (A, C or D)-1-3a
	Broughtonia sanguinea	6-nucleate	Sharp	1912	B-I-3b
	Coralliorrhiza maculata				
	Phajus grandiflorus				
	Bletia Shepherdi	occas. 6-nucl.			
	Gyrostachys gracilis	occas. 5/6-nucleate	Pace	1914	B (C or D)-I-3a (3aα)
	Gyrostachys cernua				
	Gastrodia elata	4-nucleate	Kusano	1915	B-IIa-3b

Araceae	Aglaonema commutatum	? ?	Campbell	1900, 1903, 1912	pathological
	Nephthytis Liberica	? ?	Campbell	1905	pathological
	Aglaonema pictum	5-nucleate	Campbell	1903, 1912	D-IIa-3b
	Aglaonema versicolor	6-15 nucleate	Gow	1908	? ?
	Aglaonema simplex, Aglaonema modestum	5-nucleate	Campbell	1912	D-IIa-3b

Juglans regia according to KARSTEN (1902) shows a quite normal development of the chalazal group. At the other end however only three nuclei should be formed, viz. the egg, the upper polar nucleus and one synergid, which should correspond to the formule D—IIb—1a. As the publication dates from 1902 confirmation of this condition is wanted. The more since other Juglandaceae are reported to be quite normal in this respect. Moreover the author mentions some details which he could not explain sufficiently.

First of all his statement that polars never fuse and even are often found wide apart. Secondly the fact, that the two nuclei at the micropylar end, which should represent the egg and the synergid, show no difference in size or construction. And thirdly his mentioning three cases of sacs in which, after fertilization, three dividing nuclei were seen, both nuclei at the top (synergid and egg!) still being undivided. The author's explanation is, that the second male nucleus has fused with one polar only. The figures should represent the second mitosis of this fusion-nucleus and a first division of the other unfertilized polar nucleus. A very doubtful hypothesis indeed.

Is it not safer to ascribe to Juglans regia a normal egg-apparatus? Of course this is a suggestion only, which needs verification by a renewed investigation. But KARSTEN's publication itself seems to contain rather strong arguments in its favour. It is stated that cellformation occurs very late in Juglandaceae, in Juglans nigra even not before fertilization. In my opinion the two "polars, never fusing and often wide apart" are no polars, but the egg and the fusion nucleus, Juglans regia being 8-nucleate and quite normal except as to the cell-formation of

the egg, which lies free in the sac cavity at least until fertilization. The only figure published by KARSTEN most strongly supports this suggestion, showing two absolutely equal cells (the synergids!) at the top, while the lower of the two nuclei in the sac cavity (the fusion nucleus!) is of about twice the size of the upper one (the egg). It is hardly possible to apply to the said figure KARSTEN's interpretation of one synergid and the egg at the top and of two polars in the sac. Moreover my suggestion gives a reasonable explanation of the three dividing nuclei seen after fertilization, these being the egg- and the endosperm-nucleus resp. in first and second mitosis.

Helosis guyanensis (CHODAT et BERNARD, 1900) very evidently claims the formula D—I—4α. The disintegration of the primary chalazal nucleus is already to be seen at the two-nucleate stage of the embryosac. As a rule the whole nucleus has disappeared before the micropylar's first mitosis. Only once two chalazal nuclei have been observed.

Peperomia pellucida was described by CAMPBELL as long ago as 1899 and reinvestigated by JOHNSON in 1900. CAMPBELL was quite sure about the sac being 16-nucleate but he did not succeed in tracing the further history of the sixteen nuclei after their formation. This gap however is fully filled up by JOHNSON. Up to the 16-nucleate stage both authors agree even in details. Neither in the two-nucleate stage nor in the four-nucleate one any sign of polarity or vacuolation is to be seen: "Die vier Kerne sind gleichmässig vertheilt". Not before eight nuclei are well established vacuolation commences. Very soon a large central vacuole is formed and the eight nuclei are found peripherically. A simultaneous division gives rise to the 16 nuclei of the full-grown sac. CAMPBELL supposed that afterwards three nuclei should come together at the top of the embryosac, forming the usual egg-apparatus, but he was not quite sure about their always numbering three. (His fig. S on his Plate XXXI reproduces only two nuclei). JOHNSON cleared up the matter and there can be no doubt now that there is only one synergid besides the egg. A similar group of two cells is found at the chalazal end and two other groups lie lateral. The eight re-

maining nuclei come together in the middle of the sac and fuse.

There seems to be no difficulty at all in the interpretation of the phenomena on the basis of our schemes. Vacuolation does not begin before the eight-nucleate stage, so the four-nucleate stage cannot represent anything else but four megaspores. Each of these four gives rise to a primary micropylar and a primary chalazal nucleus, and further by a second division to four nuclei two of which belong to the micropylar and two to the chalazal end. The two micropylar ones arrange themselves as egg and synergid, the chalazal ones as two polars. The mature embryosac thus contains four egg-apparatus of two cells each, and eight polar nuclei, corresponding to the formula AAAA—IIIa—3b (fig. 9, p. 43).

This conception is confirmed by JOHNSON's remark about the synergid that "the position of the spindles in certain cases seems to indicate that this is a sister to the egg" and further by BROWN's (1908) description of other Peperomia's, which all show the same development. Moreover the tendency to the reduction IIIa—3b among the Piperaceae is demonstrated by PALM (1915) mentioning abnormal sacs of this type in the usually eight-nucleate Piper subpeltatum.

Peperomia hispidula has almost the same development. According to JOHNSON (1907) however, only the micropylar egg-apparatus remains intact, while the other three "micropylar groups" do not come to the formation of cell-walls, thus leaving all nuclei free, which results in their fusing with the eight polars. In the full-grown embryo-sac only the egg, one synergid and one huge primary endosperm nucleus are left. This however does not affect the AAAA—IIIa—3b character as shown by the development.

Peperomia Sintensii and the other species described by BROWN (1908) and HÄUSER (1916) all show the same development. It is not necessary to repeat everything in detail. The tetrasporic character is emphasized by the fact that in the first division of the embryosac-mothercell of P. Sintensii a evanescent wall is formed, while "when the two nuclei divide into four, plates are formed on both spindles." Moreover in P. resediflora and

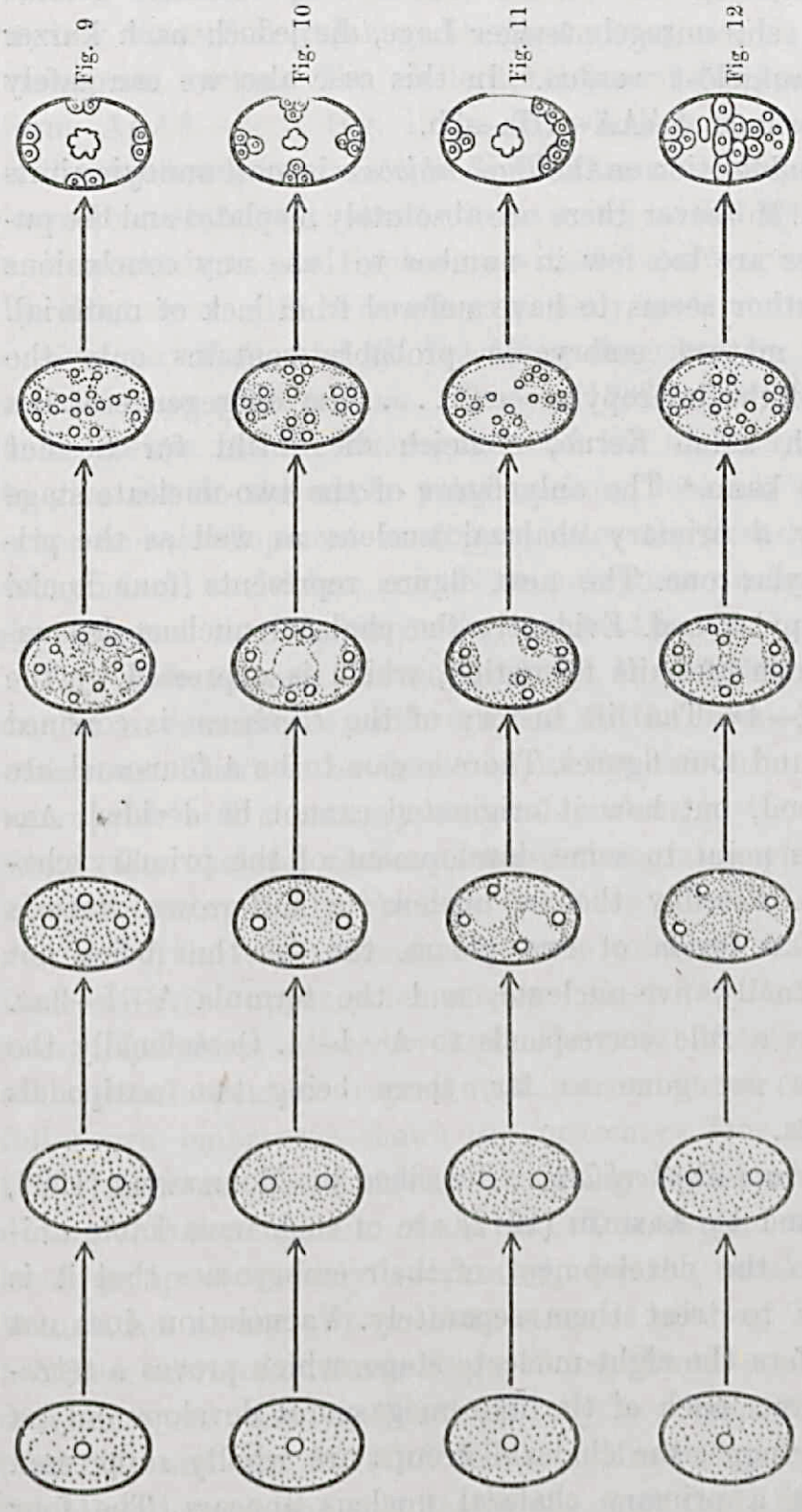


Fig. 9. *The 16-nucleate embryosac of Peperomia.* Four megaspores, each developing a micropylar and a chalazal group, both of two nuclei only. Eight fusing nuclei.

Fig. 10. *The 16-nucleate embryosac of Euphorbiaceae and Penaeaceae.* Four megaspores, all of them only developing a micropylar group. Four fusing nuclei.

Fig. 11. *The 16-nucleate embryosac of Gunnera.* Two megaspores, both fully developing. Seven fusing nuclei.

Fig. 12. *The 16-nucleate embryosac of Pyrethrum parthenifolium.* Two megaspores, both fully developing.

P. blanda "werden bei beiden Schritten der Meiosis Wände gebildet von sehr unregelmäsziger Lage, die jedoch nach kurzer Zeit wieder aufgelöst werden." In this case also we can safely accept the formula AAAA—IIIa—3b.

ARNOLDI's publication on the *Euphorbiaceae* is most annoying inits lack of detail. Moreover there are absolutely no plates and the published figures are too few in number to base any conclusions upon. The author seems to have suffered from lack of material. *Ceramanthus*' mature embryosac probably contains only the four nuclei of the micropylar end" ..... am entgegengesetzten Ende sah ich keine Kerne, obgleich dies nicht für absolut gewiss gelten kann." The only figure of the two-nucleate stage shows clearly a primary chalazal nucleus as well as the primary micropylar one. The next figure represents four nuclei at the micropylar end. Evidently the chalazal nucleus degenerates very soon after its formation, which is expressed by the formula A—I—4α. The life history of the *Codiaeum* is confined to five lines and four figures. There seems to be a four-nucleate sac at the end, but how it originated cannot be decided. ARNOLDI's figures point to some development of the primary chalazal nucleus. Possibly the sac-nucleus in full-grown state is the result of a fusion of two polars, the sac thus being not four- but actually five-nucleate, and the formula A—I—3aα. *Pedilanthus* as a rule corresponds to A—I—4. Occasionally the reduction has not gone so far, there being two antipodals left: A—I—2a.

The *16-nucleate Euphorbiaceae*, described by MODILEWSKI (1909, 1910, 1911) and by ARNOLDI (1912) are of such remarkable uniformity as to the development of their embryosacs that it is not necessary to treat them separately. Vacuolation does not commence before the eight-nucleate stage, which proves a tetrasporic condition. Each of the four megaspores develops only a "micropylar group"; the chalazal groups are wholly suppressed and not even a primary chalazal nucleus appears. The four "micropylar groups" are to be found: one at the micropylar end of the sac, one at the chalazal end and one at each end of a transverse axis. Each group organizes an egg-apparatus,

leaving one free polar-nucleus in the cavity of the sac. Ultimately these four polar nuclei fuse, giving rise to the primary endosperm nucleus. The whole development thus answers to the form. AAAA—I—5. (fig. 10, p. 43) which is in close agreement with the condition in other Euphorbiaceae. Dessiatoffs (1911) incredible statement about there being a full tetrad and still a 16-nucleate sac in Euphorbia virgata could not be confirmed by Modilewski (1911) as we can easely understand now.

Garcinia Kydia and *G. Treubii* as described by Treub (1911) and *Moringa oleifera Lam.* (Rutgers 1922) show a reduction in both groups. At the micropylar end the division, which ought to give rise to the upper polar nucleus, does not occur, at the other end development is stopped after the first division. These two chalazal nuclei fuse and act as embryosac nucleus, the whole development thus corresponding to A—IIa—3b.

The *Podostemaceae* investigated by Went (1909, 1910, 1912) are of a remarkable uniformity as to the development of the embryosac. There is no doubt about the existence of a primary micropylar and a primary chalazal nucleus in the two-nucleate stage. The chalazal nucleus soon desintegrates while the other one develops normally. Formula C—I—4α. *Lawia Zeylanica* (Magnus, 1913) is less reduced. Here the primary chalazal nucleus fuses with the upper polar nucleus: C—I—4. *Podostemon subulatus*, *Hydrobium olivaceum* and *Farmeria Metzgerioides* on the other hand seem to represent a more reduced condition. The full-grown embryosac shows the customary four nuclei, but in these cases no desorganising or fusing nuclei are to be seen during the development of the sac. It is evident that the chalazal group is entirely suppressed, for the usual polarity and vacuolation at the two-nucleate stage is missing. The difference between the two-nucleate stage of e. g. Lawia and that of Podostemon etc. can be illustrated by a comparision of fig. 9 (Taf. XI) and fig. 56 (Taf. XIV) of Magnus' publication. Also the direction of the spindles in the next (last) division leaves no doubt about the micropylar character of all four nuclei. It is true that the four nuclei are not crowded together at the top of the sac as may be seen by the Onagraceae, but the

extraordinarily small dimension makes a spreading of the nuclei through the whole of the sac inevitable. There seems to be no reason why the formula C—I—5 should not be used for these Podostemaceae. *Dicraea elongata* presents another condition. According to Magnus the mature sac consists of one synergid, the egg, and two antipodals. Only two or three of the earlier stages have been seen and a complete series could not be given. Under such circumstances it is difficult to decide about the formula. The two-nucleate stage is clearly polarised and vacuolated, and the next division would suggest also C—IIIa—3a. This however differs widely from the other Podostemaceae.

The *Penaeaceae* as far as investigated (Stephens 1908, 1909) show quite the same development as the Euphorbiaceae. The arrangement of the nuclei and the organisation of the vacuoles clearly shows a tetrasporic origin and suppression of all four chalazal groups, the formula thus being AAAA—Ia—5 (fig. 10, p. 43). The "micropylar" character of the lateral and chalazal groups of cells is emphasized by the fact that embryos were seen arising from one of these groups.

The *Onagraceae*, (Geerts 1909, Modilewski 1909, Werner 1914, Renner 1914, Täckholm 1914, 1915, Ishikawa 1918) show an absolute uniformity in their development. No need to describe the several stages in detail. The spreading of the protoplasm, the vacuolation, the direction of the spindles and the crowding of the four nuclei at the topend of the sac, these all make the total suppression of the chalazal group so evident that Geerts already said: "In der Oen. Lam. ist die erste Teilung im Embryosack ausgefallen, und es entstehen somit gar keine Antipoden und kein unterer Polkern." Form. A—I—5. He however did not recognise the individuality of the nuclei, for he still homologised the two-nucleate stage with the same stage in other Angiosperms, supposing the chalazal nucleus to be displaced by protosplasm-stream.

Of several *Gunnera* species the life history of the female gametophyte is published. We can pass the first publication (Schnegg, 1902) on the subject as his record could not be confirmed by Ernst (1908). All other publications, however, (Modi-

LEWSKI 1908, ERNST 1908, SAMUELS 1912) agree on most of the important points. The first division of the embryosac-mother-cell nucleus is not followed by wall formation and from the figures it is clear that the large vacuole is formed at the four-nucleate stage. From what is said in a previous chapter we must conclude that the Gunnera sac reprents a bisporic condition, polarisation leading us to homologize the four-nucleate stage with the two-nucleate one of normal sacs. Both megaspores of the embryosac come to full development, thus giving rise to two micropylar and two chalazal groups of 4 nuclei each. In the mature sac one of the micropylar groups (the egg apparatus) is to be found at the top, both chalazal groups come together at the bottom, while the second micropylar group, in which cell formation is omitted, fuses with the three polar nuclei of the other groups. This agrees with the figures published by the different authors, showing an ordinary egg-apparatus, six antipodals and seven fusing nuclei. Special attention might be called to the fact that this explanation of the Gunnera embryosac, based on the vacuolation, results in a reasonable explanation of the puzzling number of seven fusing nuclei. The development corresponds to CC—1—1a (fig. 11, p. 43).

Plumbago species, investigated by DAHLGREN (1915, 1916) all showed a four-nucleate sac. The two-nucleate stage is clearly polarised, both the primary micropylar and the primary chalazal nucleus giving rise to a group of two nuclei, viz. an egg, two polars and one antipodal, which corresponds to D-IIIb-3a.

Ceratostigma (DAHLGREN 1916) normally develops in the same way. Occasionally however the antipodal degenerates. In *Plumbagella* (DAHLGREN 1915, 1916) this desintegration is fixed, the full-grown sac never containing more than three nuclei: D-IIIb-3a$_z$.

Pyrethrum parthenifolium var. aureum (PALM 1915) supplies another instance of a bisporic sac. In the two-nucleate stage plasm in still homogenous, vacuolation immediately following the next division. As in Gunnera, here too, the bisporical character is accentuated by the direction of the spindles and by the early stages of vacuolation. "Bei der zweiten Teilung im

Embryosack nehmen die Spindeln eine schiefe Stellung zur Längsachse des Embryosackes ein" states PALM. This deviation from the ordinary condition and still more the vacuoles originating in two distinct groups strongly influences the character of the embryo sac. The figures themselves seem to suggest that there are two developing megaspores.

Both megaspores develop their full number of eight nuclei. Owing to the narrowness of the Pyrethrum sac the nuclei of the different groups do not mix up. The sac thus presents a row of four groups of four nuclei each, in fact a row of two eight-nucleate embryosacs.

In the upper one the nuclei are arranged in the ordinary way, there being two synergids, an egg, two fusing polar nuclei, and three antipodals. By these three antipodal cells the narrow sac is barricaded so that communication between the upper and the lower half is made impossible. In the lower sac the behaviour of the nuclei is somewhat abnormal. Four of them separated by cell walls are to be seen just below the three antipodals mentioned. The other four remain free in the sac cavity. Perhaps, however, no mature sacs are seen by the author, as PALM says: "Ueber die spätere Entwickelung dieser eigenartigen Zelle gibt leider mein Material keine sichere Auskunft." As far as present knowledge reaches the formula must be Cc—I—1a. (fig. 12, p. 43).

Pyrethrum thus closely agrees with the related Tanacetum (PALM 1915). In both the details of the development are the same, the only difference being the somewhat further reduced stage of the Tanacetum embryosac, in which only four nuclei are developed by the lower megaspore. It is almost incredible how PALM, who has been struck himself by this strong agreement, could have been so fascinated by the idea of the "16-nucleate type" that he, in spite of relationship and agreement, separated Pyrethrum from Tanacetum and classified it in the same group as Peperomia, Penaeceae, etc.

Limnocharis emarginata has been investigated twice. We can pass HALL's publication (1902) on the subject, as his improbable statement of the life-history was proved to be false by NITZSCHKE

(1914). This author describes the micropylar group as quite normal, while the primary chalazal nucleus divides only once, and sometimes twice, the six- or eight-nucleate sac thus corresponding to A—I—3a (or 1a).

Clintonia borealis' embryosac (Smith 1911) is of the Aγ—I—5 type. Here too the type is as pronounced as it was in the Onagraceae, and the resemblance to the Oenothera-sac Smith did not fail to notice. Perhaps the suppression of the chalazal group may have been induced in this case by the peculiar tetrasporical condition.

Cypripedium (Pace 1907) was one of the very first abnormal sacs discovered, and has never been reinvestigated since. Though the description is by the hand of an eminent examiner like Miss Pace, when studying the figures of her richly illustrated article the conclusion forced itself upon us that not only another more probable interpretatiou is possible, but also that Miss Pace's interpretation does not cover all the data furnished by her illustrations. The author describes the development as follows: The megaspore-mother-cell gives rise to two daughter-cells, of which the inner one (exceptionally the outer one) becomes the embryosac. (fig. 3, type C, p. 25). The primary embryosac-nucleus divides only twice. The direction of the spindle in the first division is | , of the spindles in the second divisin ⊥. In the two-nucleate stage a large central vacuole between the nuclei is to be seen. Later the four nuclei should arrange themselves as an egg-apparatus, consisting of two synergids and the egg, and one free nucleus at the bottom or halfway the embryosac. At fertilisation triple fusion should occur between this free nucleus, one of the synergids and the second male nucleus.

It is evident that a development like this cannot be explained by the thoughts which underlie this study, based as they are upon a fargoing specialising of the nuclei. The large central vacuole in the two-nucleate stage places the character of these nuclei as micropylar and chalazal beyond doubt, and so according to Pace the chalazal group should have provided the egg, to say nothing about a synergid acting as upper polar nucleus.

The remarkable way in which this review of abnormal embryosacs seems to establish our views, made us doubt Miss PACE's description and look for another explanation of the data. We will give first some facts, not in agreement with Miss PACE's view, then our own suggestion about the life history of the female gamethophyte of Cypripedium, followed by our arguments taken from PACE. Of course we cannot give more than a suggestion, a definite decision being only possible by reinvestigation of the whole material.

The objections against PACE are:

1°. The statement about there being no more than two divisions is based on the entirely negative argument that no more divisions have been seen, which is recognised by Miss PACE herself in saying: "No evidence of another division was found, although at least 300 slides with hundreds of ovules upon each were examined for this peculiar stage. When the sac is ready for fertilization, four nuclei are present, so that if other nuclei are formed they are very ephemeral". On her Plate XXIV fig. 24 she however reproduces an unfertilised, *five*-nucleate sac!

2°. The direction of the spindle in the first, and (according to PACE) only division of the micropylar nucleus is |, while the ordinary direction of the spindle in the division, which gives rise to the two synergids, is —. Moreover one of the figures (PACE, Plate XXIV fig. 26) shows the two synergids still united by fibres in — direction!

3°. The statement about the entering of a synergid in triple fusion is very poorly illustrated. As a matter of fact though "double fertilization was observed in hundreds of instances" the removal of the synergid-nucleus to the embryosac-nucleus has not been seen even once. The sacs show either, when still unfertilized, both synergids in their place at the top, or after fertilization, one synergid destroyed and two nuclei below the egg. It seems to us that Miss PACE not knowing how to trace the origin of that second nucleus, by lack of other nuclei came to the conclusion that it could be nothing else but a synergid-nucleus. This is a mere hypothesis however, and cannot be meant to be more than that; she herself with ample

material at hand, only saying that there are "two nuclei below the egg, and from the lines of cytoplasm one seems to be the synergid which has moved to that position". (PACE, Fig. 42 on Plate XXV should demonstrate these lines of cytoplasm, but in other figures e. g. 43 and 51 nothing of the kind is to be seen).

4°. It is well-known that the entering of the pollentube means the immediate destruction of the synergid. All figures of just fertilized embryosacs, published by PACE, seem to furnish proof of this occuring also in Cypripedium. On Plate XXVI, Fig. 44 she figures a synergid in which the pollentube has just entered; the nucleus of this synergid is evidently desintegrating. Her Plate XXV, fig. 43, at a little later stage, shows the two fusing nuclei at the bottom, while in the synergid there are two male nuclei and a stained thing which must be the last remnant of the synergidnucleus. Her Plate XXVI, fig. 45 represents double fertilization, while in the upper half of the embryosac the deeply stained remains of *both* synergids are to be seen.

Our own suggestion about the development runs as follows: In the two-nucleate stage there is a primary micropylar and a primary chalazal nucleus (PACE, Plate XXIV, Fig. 24). Let us follow the history of the micropylar one first. The direction of the spindle in the division of this nucleus is ⟊ (PACE, Plate XXIV, Fig. 25). But this is not the only division as PACE supposed. It is followed by a division of the upper daughter nucleus only, giving rise to the two synergid nuclei. The other nucleus remains undivided and becomes the egg (PACE, Plate XXIV, Fig. 26). As to the chalazal group only one division of the primary chalazal nucleus occurs. As a rule the prim. chal. nucleus remains undivided until just before fertilization (PACE, Plate XXIV, Fig. 26, Plate XXV, Fig. 29, 30), thus presenting a really four-nucleate embryosac. Then it divides, the two daughter-nuclei staying close together (PACE, Plate XXV, Fig. 42, 43, etc.) and acting as embryosac-nucleus. At fertilization the pollentube enters one of the synergids (PACE, Plate XXVI, fig. 44). The nucleus of this synergid at once begins to degenerate (PACE, Plate XXVI, Fig. 44). A few moments later two male nuclei have entered the synergid, whose nucleus is

rapidly shrinking (PACE, Plate XXV, Fig. 43). By the time of the fusing of the male nuclei with the egg and the double sac-nucleus, only very small remains of both synergids are still to be seen (PACE, Plate XXVI, Fig. 45). While this seems to be the ordinary course, sometimes the division of the primary chalazal nucleus may occur a little bit earlier, even at the same time of the division of the primary micropylar nucleus (PACE, Plate XXIV, Fig. 25; Plate XXV, Fig. 27; Plate XXVI, Fig. 46). Probably in these cases the fusion of both nuclei occurs already before fertilization, leaving a fusion-nucleus instead of the usual double-nucleus, as may be derived from PACE's statement that "one sac indicated the possibility that the synergid may fail to unite in the triple fusion," or in other words, that there was only one nucleus to fuse with the second male nucleus. If this record of Cypripedium proves to be right, the development of the embryosac corresponds to the formula C—IIa—3b.

Our arguments for this interpretation can be summarized as follows: 1. The insufficiency of the interpretation of Miss PACE to declare all figures given by her. 2. The details of her figures as indicated in my description. 3. The lack of evidence put forward in her arguments for the entering of a synergid-nucleus in triple fusion and for the chalazal origin of the egg, which are both entirely without analogies. 4. The analogies presented by Gastrodia, in which both chalazal nuclei fuse soon after their formation, by Garcinia, in which they fuse just before fertilization and by Moringa, in which fusion takes place after fertilization, all three showing the same trinucleate condition at the micropylar end.

As already stated we do not claim to have given a decision, this being impossible without reinvestigating the whole material. But our suggestion must be admitted as a possible explanation and must be rejected on firm grounds before we can accept Miss PACE's.

Epipactis pubescens, described by BROWN and SHARP (1911), normally has the ordinary eight-nucleate embryosac. Sometimes however the chalazal development stops at the bi-nucleate stage,

there being one polar and one antipodal nucleus. As all conditions between full tetrade and embryosac-mothercell = embryosac, occur, the formula is: B (or A, C or D)—I—1a (or 3a).

Broughtonia sanguinea, Coralliorrhiza maculata and *Phajus grandiflorus* according to SHARP (1912) have undergone some reduction in the chalazal group by omitting the last division. The only two chalazal nuclei fuse and act as lower polar nucleus. Formula B—I—3b.

Bletia Shepherdi, investigated by the same author is either normally eight-nucleate or reduced like the foregoing. Most sacs, however, showed only four nuclei, more or less fusing or desintegrating. Evidently this phenomenon can be brought back to artificial growing conditions, as the author himself has remarked.

Gyrostachys (Spiranthes) gracilis (PACE, 1914) exceptionally develops a normal eight-nucleate embryosac. Usually however the primary chalazal nucleus divides only once, giving rise to a polar nucleus and one antipodal which sometimes stays, but mostly degenerates. Formula = B (or C or D)—I—3a$_2$ (or 3a or 1a). *Gyrostachys (Spiranthes) cernua* has quite the same development, but occasionally a row of four megaspores is seen. Miss PACE referring to her figures 34 and 35 assumes the embryosac sometimes to be four-nucleate. Whether these figures really represent four-nucleate sacs might be doubted; the dimension of the sac nucleus at least points to its being a fusion nucleus. There seems to be no reason why these sacs should not be originally six-nucleate like the others. PACE's figures 31 and 36 show sacs with the antipodal gradually disintegrating, which must result in sacs like 34 and 35.

Gastrodia elata is described by KUSANO (1915) as four-nucleate like Cypripedium; the primary micropylar nucleus should produce two synergids, the chalazal nucleus the egg and the embryosac nucleus. Here too a synergid should enter in triple fusion. It is not necessary to repeat all that has been said when criticising PACE on Cypripedium. Everyone of my remarks holds for Gastrodia too. Especially the fact that KUSANO also absolutely failed to see the migration of a chalazal nucleus to

the position of the egg (his figures 93—95 which should illustrate this migration do not even give the slightest indication of a chalazal origin of the egg!) and that he too absolutely failed to see the migration of a synergid-nucleus towards the sac-nucleus, might almost be called a proof of the incorrectness of the suppossed development of Gastrodia and Cypripedium. Otherwise either PACE or KUSANO should have found one or more of the lacking stadia. Moreover KUSANO's figures so strongly resemble those of TREUB (1911) on Garcinia and so strongly suggest an explanation in that direction, that it is hard to understand why he did not come to it.

After the elaborate criticism on Cypripedium I can do with marking only a few of the most obvious phenomena in Gastrodia. First of all the fibres between the egg and synergids as illustrated in KUSANO's fig. 93 and 94, which seem to represent the ordinary behaviour and are noticed by KUSANO himself who tried to explain them by saying: "Later, the limiting plasmic membrane is precipitated between each two nuclei, often preceded by the formation of fibres." To me it seems more reasonable to accept a micropylar origin of the egg and to do without the far-fetched explanation of the fibres. A second phenomenon, which makes a chalazal origin of the egg not only improbable but quite impossible is illustrated in fig. 88, 89, 90 and 91. Though KUSANO himself says "it is almost customary that they (viz. the chalazal nuclei) lie in close contact (fig. 91)," he does not hesitate to consider the *majority* of his material as abnormal! According to him the growth and division of the chalazal nucleus should be much disturbed by the lesser amount of cytoplasm, and all those sacs shoulds be unable to come to full development. I do not think such a presumption can be accepted unless every other possibility is at least tested and rejected on firm grounds.

As far as can be gathered from KUSANO's publication the development seems to be as follows: The embryosac (one of a row of three megaspores) is in its bi-nucleate stage clearly polarized (KUSANO, fig. 78, 79, 81, 86, etc.). The primary micropylar nucleus presents a reduced development, giving rise

to two synergids and the egg (KUSANO, figg. 93, 94). The division which should give rise to the egg and upper polar nucleus is suppressed, there thus being no upper polar nucleus. The primary chalazal nucleus on the other hand divides only once. As a rule both nuclei fuse soon after their formation (KUSANO, fig. 89, 90, 91). Sometimes however this fusion may be a little bit postponed (KUSANO fig.85). Only two of KUSANO's figures do not agree with this suggestion. Both figures (80 and 84) represent nuclear divisions which by the direction of their spindles and by the distribution of the plasm point to sacs of the type B—I—5, instead of B—IIa—3b. I must emphasize here that of course it is not possible to give a description of a life history without any material at hand. Only a thorough reinvestigation can clear up the matter.

Aglaonema commutatum has been studied by CAMPBELL (1900, 1903, 1912). He did not succeed, however, to give an idea of the development. All his material of this species was collected from plants grown under more or less artificial conditions, and shows the common pathological phenomena like indefinite number of nuclei, multiple fusions and other abnormalities. Moreover "there is some evidence that the complete embryosac may be the product of the union of several sporogenous cells (megaspores)."

Nephthytis liberica is not better known (CAMPBELL, 1905). It was „quite impossible to make out any prevailing type", and as this material too came from the greenhouse, the author rightly remarks: "How far these are normal cannot be certainly determined until material grown under natural conditions can be examined." More stress is still laid on the presumption of pathological conditions by the fact that "the pollen grains were badly shrunken and distorted, and no satisfactory study .... could be made."

Aglaonema pictum (CAMPBELL 1903, 1912) was first assumed to have a normal eight-nucleate embryosac. In his later publication CAMPBELL however describes the sacs as five-nucleate, the type being D—IIa—3b.

Aglaonema versicolor (GOW, 1908) should be 6 to 15-nucleate

There should be a normal development of the micropylar group, while at the other end a different number of nuclei, varying from 2 till 11, should be produced. Gow however has not seen all stadia of development. His record of what he has seen is very brief and the figures very few in number. Moreover these figures are only outlined and of no use for further research.

Aglaonema simplex and *A. modestum* (Campbell, 1912) are five-nucleate and of the D—IIa—3b type.

V. CONCLUSIONS REGARDING SYSTEMATICS AND PHYLOGENY.

Our knowledge of the embryosac development of most families is yet too scanty to justify any attempt at an exact system of the female gametophyte. The survey however still indicates certain tendencies in development and it furnishes some unexpected evidences of relationship worth special mention.

Among *Piperaceae* Peperomia shows a regular development of four megaspores and a regular reduction in the number ot nuclei produced by each of these megaspores. Exactly the same tendencies are occasionally met with in Piper. It is only by analising gametophytes as we did, that this relationship between a 16- and 5-nucleate sac came to light.

Euphorbiaceae may be either 16-, 8-, 7-, 5- or 4-nucleate. Superficially any connection between these types seems to be lacking. In fact, however, they are as closely related as possible. Only the chalazal group of nuclei is affected by a process of reduction, which can go as far as total suppression. In the 16-nucleate sacs this process is combined with the development of all four megaspores.

In *Penaeaceae* the same combination of total suppression of the chalazal group with a tetrasporical condition is to be seen.

All *Onagraceae* are like the Penaeaceae, except their developing only one of the four megaspores.

Monocotyledones show a great variety of types. Reduction processes are still going on in every direction. The number of megaspores ranges from four to one, and both the micropylar

and the chalazal group may suffer from a decrease in the number of nuclei. Among Orchidaceae these divergent lines of development are even met with in the same family. In some of the species the micropylar group is still quite normal, the chalazal one being affected by reduction in the number of nuclei; in other species the micropylar end too never reaches the four-nucleate stage.

Our method of treating the various sections (megaspore-formation — micropylar group — chalazal group) of the gametophyte as morphological units, capable of following independent lines of development, proved to be a real progress, in so far at least as closely related species are no longer scattered all over a system. In this respect our outlines show distinct advantages on COULTER'S, ERNST'S, DAHLGREN'S and ISHIKAWA'S schemes.

Especially the application of the idea to the 16-nucleate embryosacs has been fruitful. These sacs are no longer a type of their own, but are considered as either of bi- or of tetrasporical origin. A fully developed tetrasporical sac should be 32-nucleate, each megaspore developing a "micropylar" and a "chalazal" group. Both however can be subject to reduction conformable to the possibilities, worked out and illustrated in figures 5—7. Several of these reduction types can be 16-nucleate. Of course full development of a bisporical sac also leads to a 16-nucleate embryosac.

Up to the present moment the Angiosperm embryosac has held a wholly isolated position. Its origin could not be traced and its analogies among Gymnosperms were dark. A gap in phylogenetical knowledge on such an important point, necessarily has led to numerous suggestions. None of these, however, passed the hypothesis stage. It apparently depends in the main on the author's preference for a certain theory on the origin of Angiosperms, which type of embryosac he will call the most primitive one. It must be admitted that this is not the right way of settling the question, using phylogenetical speculations

as basis for a system, instead of systematics as basis for phylogeny.

The present study did not succeed in throwing more light on the origin of the Angiosperm sac. It has led us to the view that all embryosacs with an abnormal number of nuclei are derived from the normal eight-nucleate type. This conception of the 8-nucleate sac as the most primitive one fully agrees with the actual conditions, for it is met with in all families at the bottom of the natural system. On this point the phylogenetical value of our results is purely negative: it leads us to reject all theories on the origin of Angiosperms, which are founded on the "primitive character" of the 16-nucleate embryosac or of the embryosac with an increased number of antipodals.

VI. FACTORS WHICH MAY ACCOUNT FOR THE ANOMALIES IN EMBRYOSAC-DEVELOPMENT.

The reduction in the number of megaspores ("row of four", "row of three", "row of two", "no row at all"), in the number of micropylar nuclei (four, three, two or one nucleus), in the number of chalazal nuclei (four, three, two, one or no nucleus), they all succesfully can be brought back to the same causes.

First of all the process of shortening the sex generation must be mentioned. In most Angiosperms the sporogenous tissue has been reduced to one cell only. So the next step on this way necessarily must affect megaspore-formation and embryosac-development.

Secondly there is the usual desintegrating and final suppression of non-functioning tissues. This is too well-known from sporophytic conditions to need any further commentary. Its application to the megaspore-formation and embryosac-development will meet no objections.

The reduction in the number of megaspores probably is caused by both processes. Normally three of the spores are seen desintegrating. A total suppression should be an anticipation on this degeneration.

The reduction at the chalazal end too may be influenced by

both factors. Most authors who have made special study of the subject agree on ascribing to the antipodals a nutritive function (Westermaier 1892, Ikeda 1902, Lötscher 1905, Huss 1906). Their losing this function leads to desintegration and finally to total omission. This, of course does not hold for the lower polar nucleus. Still this nucleus may be suppressed too, which shows that the process of shortening the sex-generation is at work as well.

At the micropylar end the nuclei, once formed, usually persist. They have all got a special function. The occasional suppression of one or two of these nuclei therefore must be considered as a result of the shortening of the n-generation. This conception explains why reduction in the number of megaspores and in the number of chalazal nuclei is more common than in the number of micropylar nuclei. The development of the micropylar group is affected by one reducing factor only, while in megaspore-formation and chalazal development two factors are at work.

An increase in the number of chalazal nuclei, on the other hand, safely can be ascribed to a more intensive nutritive function. This view is supported by Campbell's (1899a, 1899b) statement about the occasional increase after fertilization and in relation to the nourishing of the embryo.

VII. ABNORMAL SACS WHICH SHOULD FAIL TO FOLLOW THE OUTLINED SYSTEM.

We have to mention here some literature about embryosacs, showing special anomalies not in keeping with the results of the present study.

First of all Campbell's publications on Aglaonema commutatum (1900, 1903, 1912) and on Nephthytis liberica (1905). We have already cited these cases in our general review. The irregularities are doubtless pathological and caused by the abnormal conditions under which the material was grown (in greenhouses). Especially the many multiple fusions strongly remind us of Němec's studies on the influence of external circumstances on nuclear division and nuclear fusions.

Secondly three cases of two micropylar eggs are reported (Strasburger 1878, Fischer 1880, Murbeck 1902). In itself a secondary increase in the number of micropylar nuclei is not in opposition to our views. In more recent literature however no such cases are met with. Therefore these records were not inserted in our general survey, as they can only be accepted under reserve of further confirmation. Especially since these cases do not represent normal conditions, but anomalies. Fischer himself even doubts the correctness of his observation, the only indication being *one* section „dessen Tauglichkeit durch den Schnitt leider herabgesetzt worden ist."

Thirdly in a few publications a synergid is mentioned as having assumed the function of an egg or of an upper polar nucleus. Almost all of these studies are dated long before triple fusion was known. The only exceptions are Pace on Cypripedium and Kusano on Gastrodia, but their figures probably have been misinterpreted, as we have already discussed (p. 49—52, 53—55).

Lastly we have to deal with four cases in which there should be an egg of chalazal origin (Chamberlain 1895, Tretjakow 1895, Pace 1907 and Kusano 1915). There is no need to repeat again whath has been said in our discussion on Cypripedium (Pace) and on Gastrodia (Kusano). As to the other two: Marie Oppermann (1904), when reinvestigating the Aster embryosac, says that "there was nothing to indicate the presence of an antipodal egg". She too noticed that often one of the antipodals becomes larger than the other two, "but in no instance was I able to find in this lowest cell an antipodal oosphere as described by Chamberlain (1895)". The embryosac of Allium odorum (Tretjakow, 1895) is of the normal 8-nucleate type. The author speaks of embryo formation "zuweilen sogar aus allen drei Antipoden, und zwar ohne Befruchtung." This development begins "erst nach der Befruchtung der Eizelle". This really seems to be nothing else but a secondary increase of antipodal nuclei, quite common now.

We seem justified in finishing this study by stating that all literature on the Angiosperm embryosac confirms our views, the only exceptions being: a few publications of too old a date

to be accepted without further confirmation, and two more recent studies (PACE, 1907 and KUSANO, 1915) in which however figures are probably misinterpreted.

VIII. SUMMARY.

1. Several attempts have been made to classify the various types of Angiosperm embryosacs. These systems are based either on the number of divisions between embryosac-mothercell and egg (COULTER) or on the number of nuclei in the full-grown sac (ERNST). They are wholly artificial and therefore without any phylogenetical value.
2. The female gametophyte is no morphological unit, but a complex, as well as the sporophyte. A natural system therefore presupposes thorough and detailed knowledge of morphology. It has to reckon with the following processes as probably independent lines of development:
   Chromatine reduction.
   Megaspore formation.
   Polarisation.
   Development of a micropylar group of nuclei.
   Development of a chalazal group of nuclei.
3. *Chromatine reduction* usually accompanies the first divisions of the embryosac-mothercell. Sometimes (in apogamous species) it is omitted, which proves that it is not identical with megaspore-formation.

   Polarisation is a function of the developing megaspore (embryosac). It does not accompany megaspore-formation, but megaspore-development. It commences as soon as megaspore-development begins. It therefore provides us with means of recognising megaspores, even when two or four megaspores are lying in the same cell: as long as plasm remains homogenous spore-formation is still going on, as soon however as polarisation (vacuolation) commences we have to do with germinating spores. Moreover a large central vacuole enables us to tell the nuclei of the chalazal group from those of the micropylar group.

Megaspore formation usually leads to a "row of four." Occasionally however only three or two megaspores are formed, or even the embryosac-mothercell itself is seen functioning as a megaspore. The omission of cell walls during spore formation may affect the number of nuclei in the mature embryosac: four germinating megaspores in the same cell give rise to a tetrasporical, three to a trisporical and two to a bisporical sac. We are forced to admit theoretically: 9 types of monosporical, 5 of bisporical, 2 of trisporical and 1 of tetrasporical sacs. The further development of the megaspores must be considered as wholly independent from their formation.

Development of the micropylar group of nuclei. Normally it results in a group of four nuclei. Sometimes however the number has been reduced. Theoretically this reduction can go down to there being only one nucleus left, the primary micropylar nucleus or even the megaspore itself thus assuming the egg-function.

Development of the chalazal group of nuclei. Here all stages of the reduction series may be met with, from the usual four down to total suppression of the entire group. Even when nuclei are still formed their desintegration is of common occurence. On the other hand a secondary increase in the number of nuclei sometimes has been reported.

4. A few, most probably incorrect, records excepted, all publications on abnormal embryosacs seem to confirm this conception of considering the Angiosperm embryosac as a morphological *complex*.
5. Another confirmation is to be found in its systematical value. Relationships are established by the study of the gametophyte as well as by the study of the sporophyte. Especially the application of our views to 16-nucleate sacs has been fruitful. They are no longer a type of their own, but of bi- or tetrasporical origin, each one of their megaspores being open to the deviations, which we have worked out with regard to the monosporical sac.
6. The reduction processes by which megasporeformation, micro-

pylar- and chalazal development occasionally are affected, can be traced back to two causes, viz. the shortening of the sex-generation and the usual desintegration and final suppression of non-functioning tissues. Megaspore-formation and chalazal development are attacked by both factors, micropylar development by the first one only.

7. The 8-nucleate sac seems to be the most original type of the Angiosperm embryosac. The present study did not succeed in throwing any light on its origin.

LITERATURE.

ARNOLDI, W. (1912). Zur Embryologie einiger Euphorb. Trav. du Musée Bot. de l'Acad. Imp. d. Sc. St. Petersb. IX.

BROWN, W. H. (1908). The nature of the embryosac of Peperomia, Bot. Gaz. 46.

——, (1909). The embryosac of Habenaria, Bot. Gaz. 48.

BROWN, W. H. and L. W. SHARP. (1911). The embryosac of Epipactis, Bot. Gaz. 52.

CAMPBELL, D. H. (1899*a*). Die Entw. des Embryo sackes von Peperomia pellucida Kunth, Ber. d. D. Bot. Ges. 17.

——, (1899*b*). Notes on the structure of the embryosac in Sparganium and Lysichiton, Bot. Gaz. 27.

——, (1900). Studies on the Araceae, Ann. of Bot. 14.

——, (1902). Recent invest. upon the embr.sac of Angiosperms, Amer. Naturalist 36.

——, (1903). Studies on the Araceae; the embr. sac and embryo of Aglaonema and Spathicarpa, Ann. of Bot. 17.

——, (1905). Studies on the Araceae III, Ann. of Bot. 19.

——, (1908). The embryosac of Pandanus, prel. note, Ann. of Bot. 22.

——, (1909). The embryosac of Pandanus, Bull. Torrey Bot. Club 36.

——, (1910). The embryosac of Pandanus coronatus, Bull. Torrey Bot. Club 37.

——, (1911). The embryosac of Pandanus, Ann. of Bot. 25.

——, (1912). The embryosac of Aglaonema, Scott. Bot. Rev. April 1912.

CANNON, W. A. (1900). A morph. study of the flower and embr. of the wild Oat (Avena fatua L.), Proc. Calif. Acad. Sc. 3d ser. Bot. Vol. I.

CHAMBERLAIN. CH. J. (1895). The embryo sac of Aster Novae-Angliae, Bot. Gaz. 20.

——, (1905). Alternation of generations in animals from a botanical standpoint, Bot. Gaz. 39.

CHODAT R. et BERNARD C. (1900). Sur le sac embr. de l'Helosis guyanensis, Journ. d. Bot. 14.

COULTER, J. M. (1908). Relation of Megaspores to Embryosacs in Angiosperms, Bot. Gaz. 45.

COULTER, J. M. and CHAMBERLAIN, CH. J. (1912). Morphology of Angiosperms, New York 1912.

DAHLGREN, K. V. O. (1915). Der Embr. sack von Plumbagella, Arkiv. f. Botanik 14.

——, (1916). Zytol. und Embryolog. Studien über die Reihen Primulales und Plumbaginales, Kungl. Sv. Vetensk. akad. Handl. 56.

DAVIS, (1905). Studies on the plant cell, Amer. Naturalist 38.

DESSIATOFF, N. (1911). Zur Entw. des Embr. sackes von Euphorbia virgata W. R., Ber. d. D. Bot. Ges. 29.

ERNST, A. (1902). Chromos. red., Entw. des Embr. sackes und Befr. bei Paris quadrifolia L. und Trillium grandiflorum Salisb., Flora 91.

——, (1908*a*). Zur Phylog. des Embr. sackes der Angiosp. Ber. d. D. Bot. Ges. 26*a*.

——, (1908*b*). Ergebnisse neuerer Unters. über den Embryosack der Angiosp., Verh. d. Schweiz. naturforsch. Ges., 91 Jahres-Vers., Glarus 1908, Band I.

FISCHER, A. (1880). Zur Kenntniss der Embryosackentwicklung einiger Angiospermen, Jenaische Zeitschr. 14.

Geerts, J. M. (1909). Beitr. zur Kenntn. der Cytol. und der part. Sterilität von Oenothera Lamarckiana, Rec. d. Trav. Bot. Néerl. 5.

Gow, J. E. (1908). Studies in Araceae, Bot. Gaz. 46.

Hall, J. G. (1902). An embryological study of Limnocharis emarginata, Bot. Gaz. 33.

Häuser, R. (1916). Untersuchungen an Makrogametophyten von Piperaceën, Beitr. zur Allg. Botanik, 1.

Huss, H. A. (1906). Beitr. zur Morph. und Physiol. der Antipoden, Beih. z. Bot. Centr. bl. 20.

*Ikeda, (1902). Studies in the physiol. functions of Antipodals, Bull. Coll. Agric. Imp. Univ. Tokyo 5.

Ishikawa, M. (1918). Studies on the Embr. sac and Fertilization in Oenothera, Ann. of Bot. 32.

Jacobsson-Stiasny, Emma. (1916). Fragen vergleichender Embryologie der Pflanzen, Sitz. ber. Kais. Ak. d. Wiss. Wien, Abt. I, Bd. 125.

Johnson, D. S. (1900). On the endosperm and embryo of Peperomia pellucida, Bot. Gaz. 30.

——, (1907). A new type of embryo-sac in Peperomia, Johns Hopkins Univ. circ. no. 195 (1907, 3).

——, (1914). Studies on the developm. of the Piperaceae II. The structure and seed-developm. of Peperomia hispidula, Amer. Journ. of Bot. I.

Karsten, G. (1902). Ueber die Entw. der weibl. Blüthen bei einigen Juglandaceen, Flora 90.

Kusano, S. (1915). Exp. Studies on the Embr. developm. in an Angiosp., Journ. Coll. of Agric. Imp. Univ. Tokyo 6.

Lötscher, P. K. (1905). Ueber den Bau und die Funktion der Antipoden in der Angiosperm.-Samenanlage, Flora 94.

Magnus, Werner. (1913). Die atypische Embr. Entw. der Podostemaceen, Flora 105.

Mc. Allister, F. (1909). The developm. of the embr.sac of Smilacina stellata, Bot. Gaz. 48.

——, (1914). The developm. of the embr.sac in the Convallariaceae, Bot. Gaz. 58.

Modilewski, J. (1908). Zur Embryobildung von Gunnera chilensis, Ber. d. D. Bot. Ges. 26*a*.

——, (1909*a*). Zur Embryobildung von Euphorbia procera, Ber. d. D. Bot. Ges. 27.

——, (1909*b*). Zur Embryobildung von einigen Onagraceen, Ber. d. D. Bot. Ges. 27.

——, (1910). Weitere Beitr. zur Embryobildung einiger Euphorbiaceen, Ber. d. D. Bot. Ges. 28.

——, (1911). Ueber die anomale Embr.sackentw. bei Euphorbia palustris L. und und anderen Euphorbiaceen, Ber. d. D. Bot. Ges. 29.

Murbeck, Sv. (1901). Parthenogen. Embryobildung in der Gattung Alchemilla, Lunds Univ. Årsskr. 36, 2, No. 7.

——, (1902). Ueber die Anomalien im Baue des Nucellus und des Embryosackes bei parthenogen. Arten der Gattung Alchemilla, Lunds Univ. Årsskr. 38.

Němec, B. (1910). Das Problem der Befruchtungsvorgänge und andere zytol. Fragen, Berlin 1910.

Nitzschke, Joh. (1914). Beitr. zur Phylog. der Monokotylen, gegründet auf der Embr.sackentw. apokarp. Nymphaeaceen und Helobien, Beitr. z. Biol. der Pflanzen 12.

Opperman, Marie (1904) A contribution to the life history of Aster, Bot. Gaz. 37.

Pace, Lula. (1907). Fertilization in Cypripedium, Bot. Gaz. 44.

——, (1909). The Gametophytes of Calopogon, Bot. Gaz. 48.

——, (1914). Two species of Gyrostachys, Baylor Univ. Bull. 17.

Palm, B. (1914) Ueber die Embr. Entw. einiger Kompositen, Sv. Bot. Tidsskr. 8.

——, (1915). Studien über Konstruktionstypen und Entwickelungswege des Embryosackes der Angiospermen, Inaug. Diss. Stockholm 1915.

Renner, O. (1914). Befr. und Embr. bild. bei Oenothera Lam. und einigen verw. Arten, Flora 107.

Rutgers, F. L. (1922). Embryosac and Embryo of Moringa oleifera Lam., Ann. d. Jard. Bot. Buitenzorg 32.

Samuels, J. A. (1912). Etudes sur le développement du sac embr. et sur la fécondation du Gunnera macrophylla Bl., Arch. f. Zellforschung 8.

Schnegg, Hans (1902). Beitr. zur Kenntniss der Gattung Gunnera, Flora 90.

Schniewind-Thies, J. (1901). Die Redukt. der Chromos. zahl und die ihr folg. Kernteilungen in den Embr. s. mutterzellen der Angiosp., Jena 1901.

Schürhoff, B. N. (1919). Zur Phylogenie des Angiospermen Embryosackes, Ber. d. D. Bot. Ges. 37.

Sharp, L. W. (1912). The Orchid Embryosac, Bot. Gaz. 54.

Smith, R. Wilson (1911). The tetranucleate embryosac of Clintonia, Bot. Gaz. 52.

Stephens, E. L. (1908). A prelim. note on the embryosac of certain Penaeaceae, Ann. of Bot. 22.

——, (1909*a*). The embr. sac and embryo of certain Penaeaceae, Ann. of Bot. 23.

——, (1909*b*). Recent progress in the study of the embr. sac of the Angiosp., New Phytol. 8.

Strasburger, Ed. (1878). Ueber Befr. und Zelltheilung.

Täckholm, G. (1914). Svensk Bot. Tidsskr. 8.

——, (1915). Svensk Bot. Tidsskr. 9.

Tretjakow, S. (1895). Die Betheiligung der Antipoden in Fällen der Polyembryonie bei Allium odorum L., Ber. d. D. Bot. Ges. 13.

Treub, M. (1911). Le sac embr. et l'embryon dans les Angiospermes, Ann. d. Jard. Bot. Buitenzorg 24.

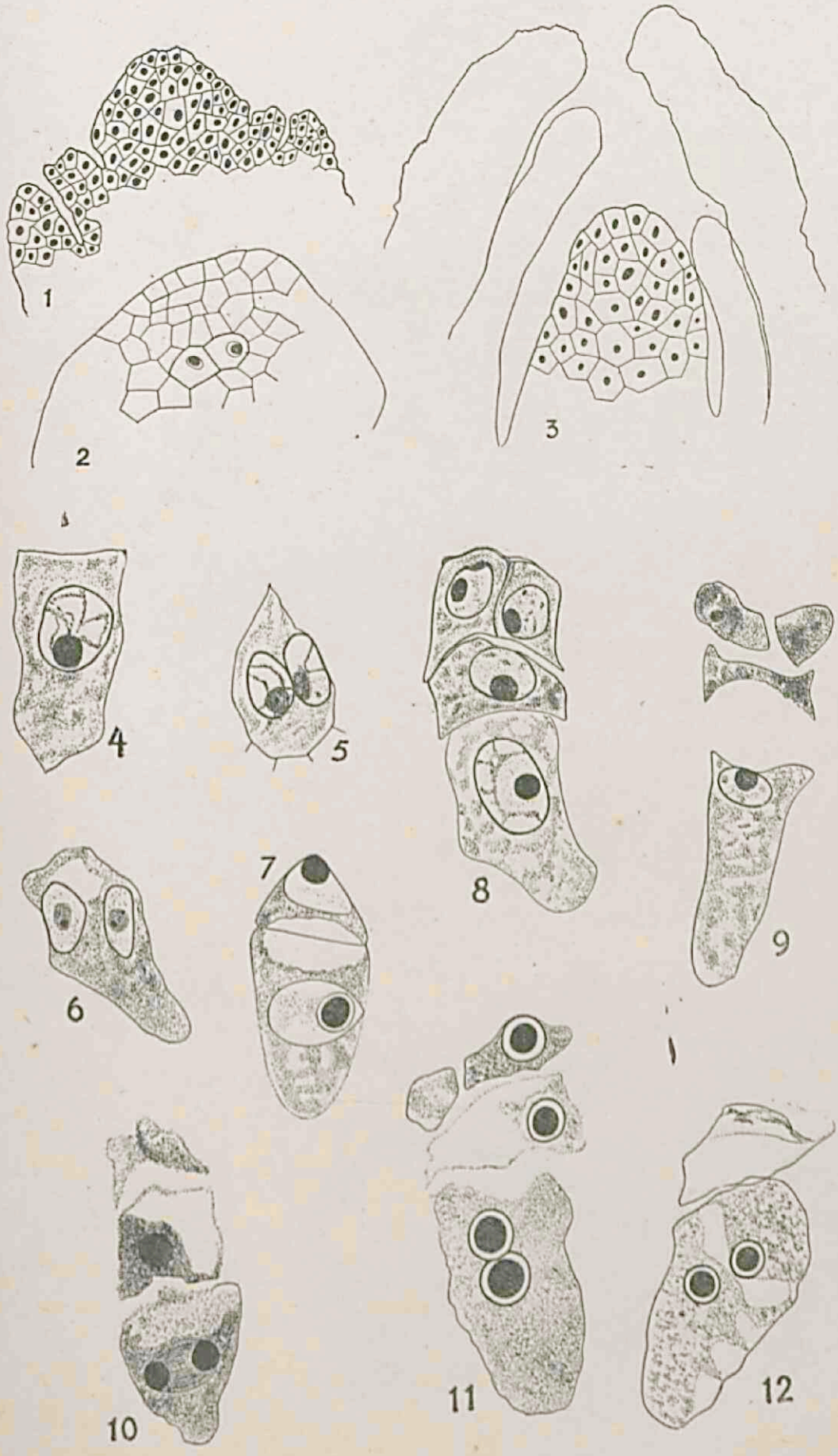
Went, F. A. F. C. (1908). The development of the ovule, embryosac and egg in Podostemaceae, Rec. Trav. Bot. Néerl. 5.

——, (1910). Unters. über Podostemaceae, Verh. Kon. Akad. Wet. Amsterdam 16.

——, (1912). Unters. über Podostemaceae II, Verh. Kon. Akad. Wet. Amsterdam 17.

Werner, Elisabeth (1914). Zur Oekologie atypischer Samenanlagen, Beih. z. Bot. Centr. bl. 32.

Westermaier, M. (1892). Zur Embryologie der Phanerogamen, insbesondere über die sogen. Antipoden, Nova Acta Acad. Leopold. Carol. 57.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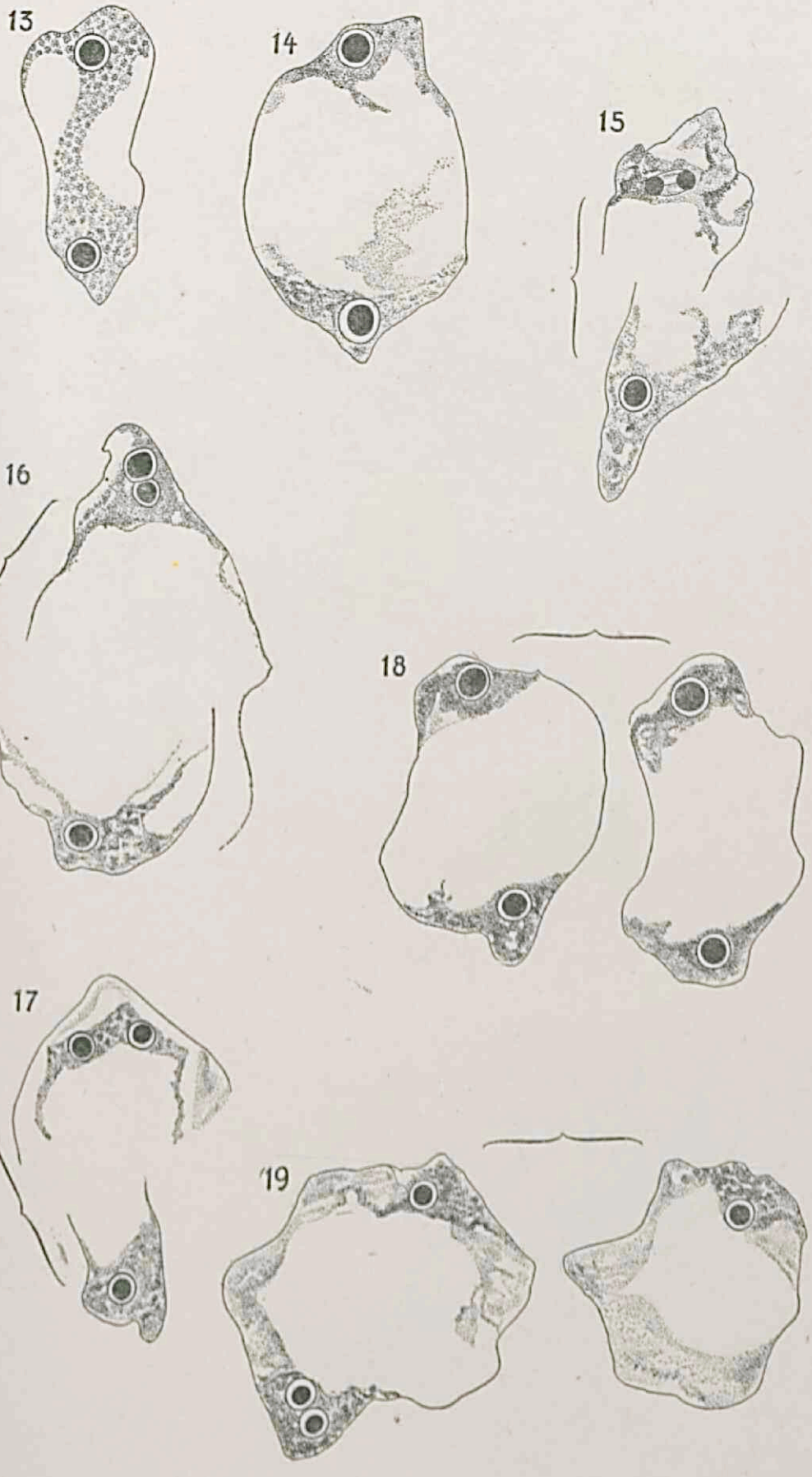
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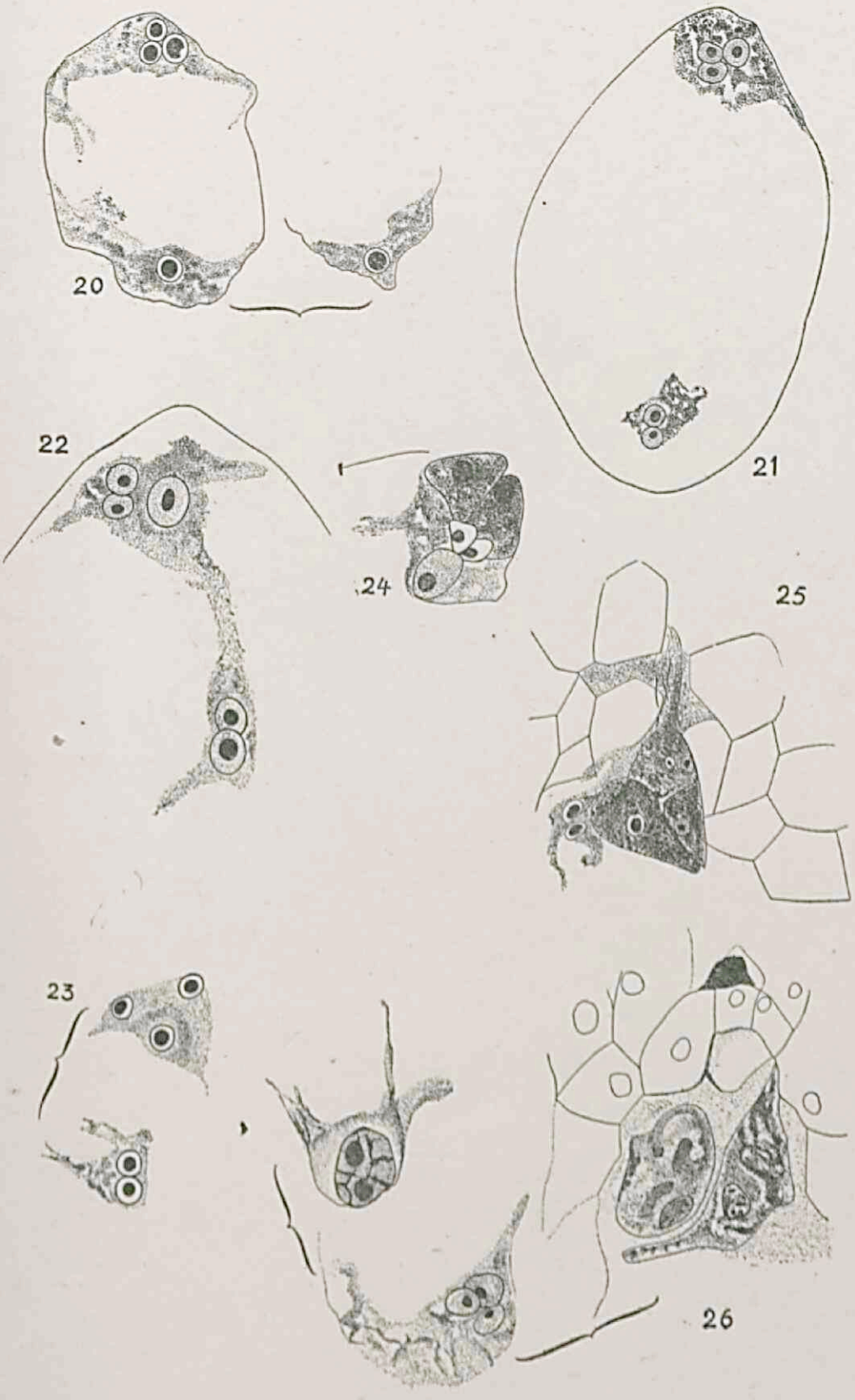
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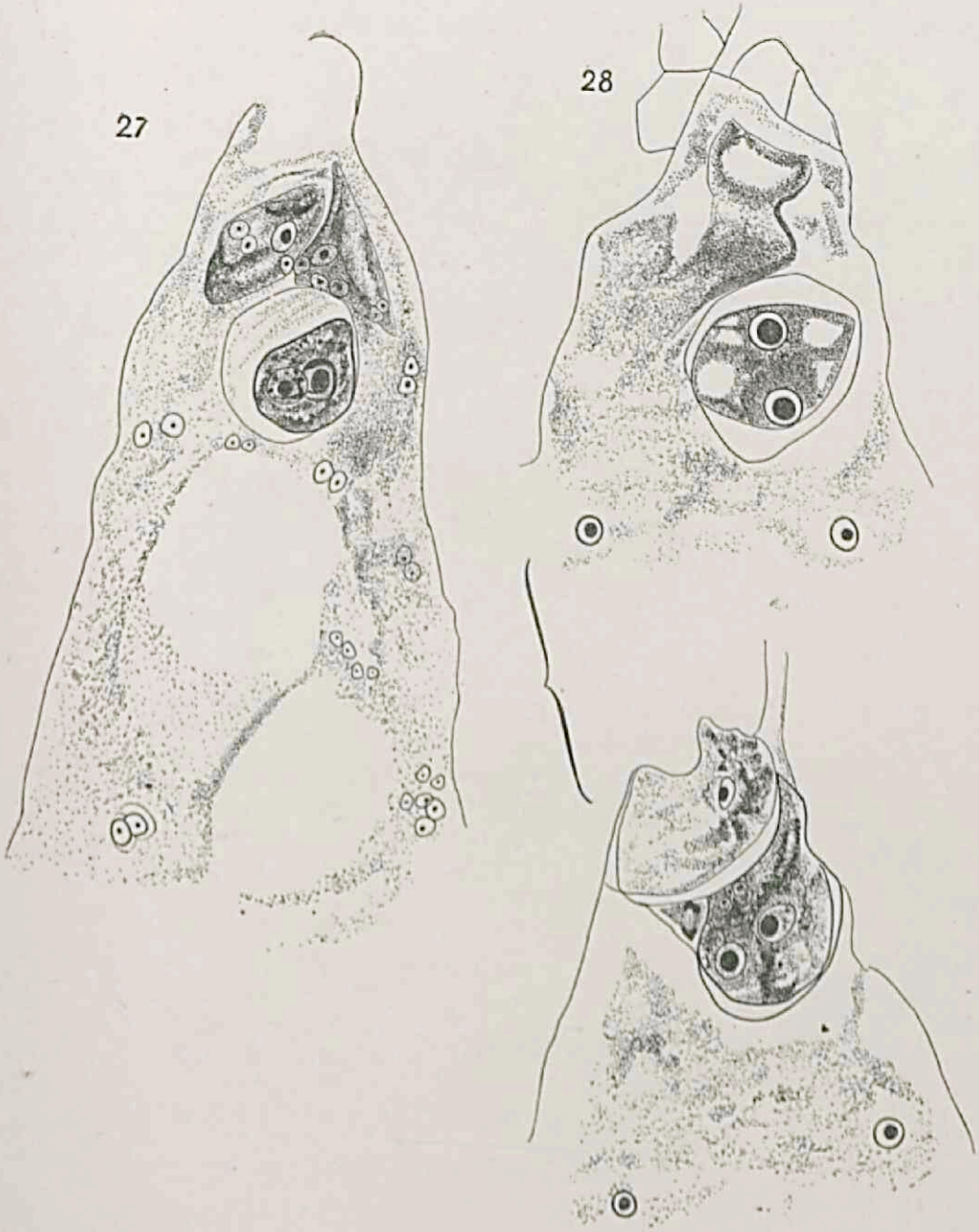
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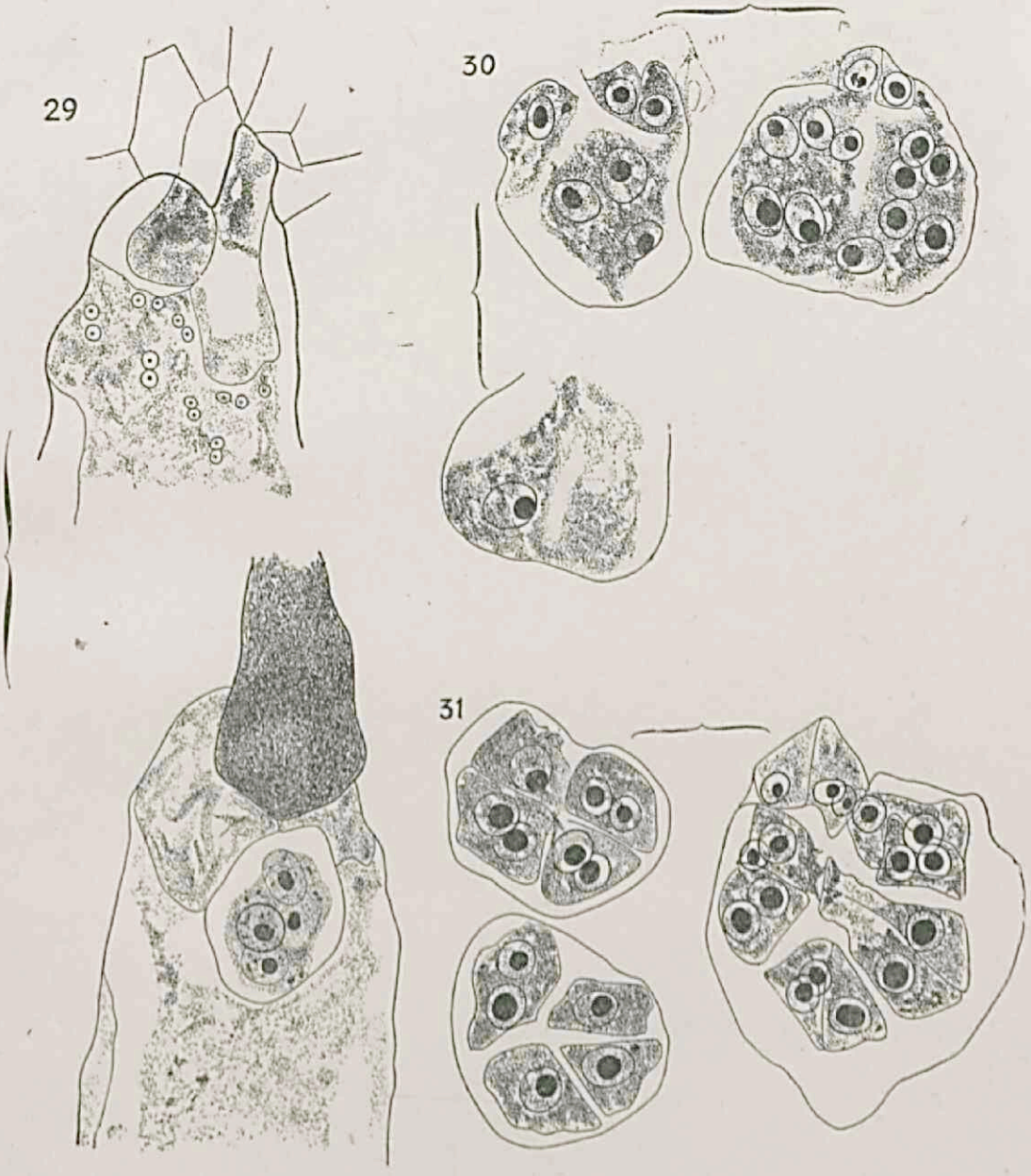
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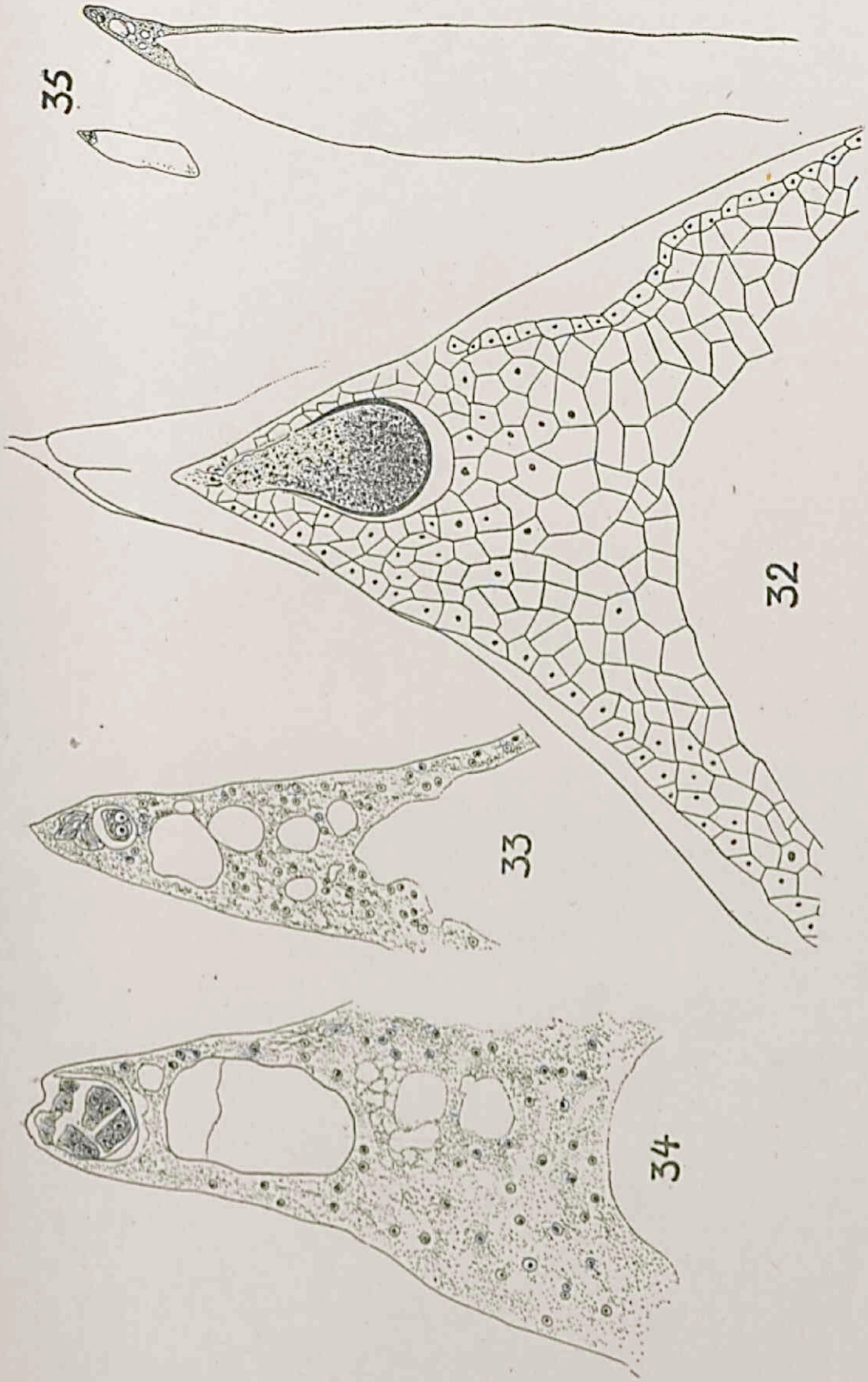
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STELLINGEN.

STELLINGEN.

I.

Voor een „natuurlijk einde" van leven bestaan geen bewijzen. Veeleer moet een physiologische onsterfelijkheid worden aangenomen.

II.

Aan MORGAN's crossing-over theorie en zijn opvatting van een groepsgewijze lineair gebonden zijn der dragers van de erfelijke eigenschappen, kan slechts de waarde van een werkhypothese worden toegekend.

III.

De oorsprong van Zea Mays is niet te zoeken in Zea tunicata, maar in een bastaard tusschen Euchlaena en een der Andropogoneae.

IV.

De exostichos en endostichos, die BOLK bij Zoogdieren beschrijft, zijn niet homoloog met de stichi der Reptielen, volgens de definitie van WOERDEMAN.

V.

De Foraminiferen vormen geen natuurlijke groep. Het is beter de Monothalamia met de Amoebozoa tot één orde te vereenigen en de Polythalamia als een afzonderlijke orde te beschouwen.

VI.

De opvatting, dat de löss een IJstijdvorming is, staat sterker dan de meening, welke haar een interglacialen ouderdom toeschrijft.