SOME ASPECTS IN THE EMBRYOLOGY OF ZYGOGYNUM BAILLONI V. TIEGH.

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The family Winteraceae occupies a rather significant position—especially on account of its vesselless xylem and the remarkably complete series of stages that its members present in the phylogenetic closure of the unsealed conduplicate carpel—in studies concerning the phylogeny of angiosperms. The recent investigations of Prof. I. W. Bailey and his associates (see Bailey and Nast, 1945, for a summary and literature) have greatly enlightened our knowledge in regard to the anatomy and morphology of the vegetative structures of the family. However, very little is known as to the internal development and organisation of the reproductive structures. The casual observations of Willie (1886) on the pollen of *Drimys Winteri*, of Strasburger (1905) on the development of the ovule, and of Bhagavathi Kutti Amma (1938) on the microsporogenesis of the same species are the only contributions towards the embryology of the family.

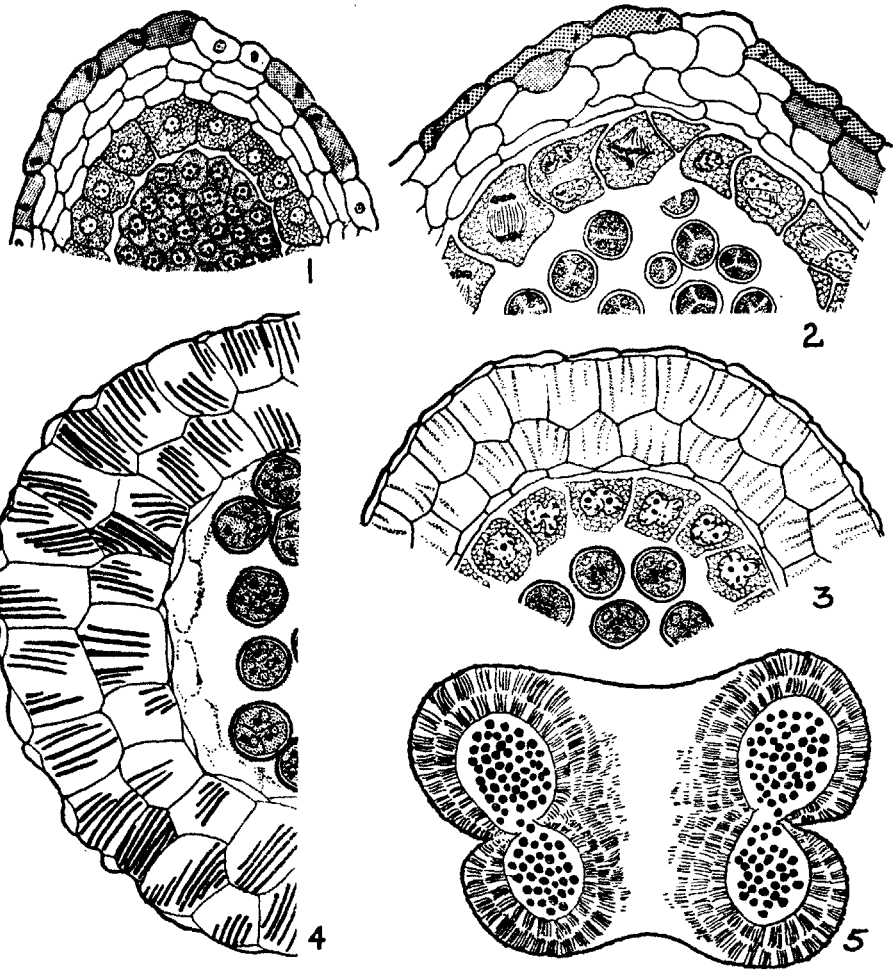
The genus *Zygogynum* with its six recognised species is endemic in New Caledonia. In 1948, the late Prof. J. T. Buchholz visited this island on a plant collection trip and brought back with him a small quantity of flowers and maturing fruits fixed in FAA. This material was forwarded to Prof. I. W. Bailey of Harvard University and has formed the basis for present study.

OBSERVATIONS.

Microsporogenesis.—The wall of the youngest anthers available to me shows an epidermis, several cells of which are filled with brownish phenolic compounds, three subepidermal layers of parenchymatous cells, and an innermost layer of larger tapetal cells containing minutely vacuolate, granular-appearing, dense cytoplasm with a centrally situated nucleus. The interior of the loculus is occupied by a rather massive aggregation of microspore mother cells (Fig. 1). In the course of maturation, the epidermis as well as the wall layer lying immediately outside the tapetum become almost disintegrated, whereas the two layers of cells in between these enlarge, essentially in a radial direction, and develop the characteristically banded thickenings of the endothecium (Figs. 3, 4). In still older stages the wave of differentiation of the endothecial thickenings spreads to two to four more layers of cells towards the interior of the anther (Fig. 5).

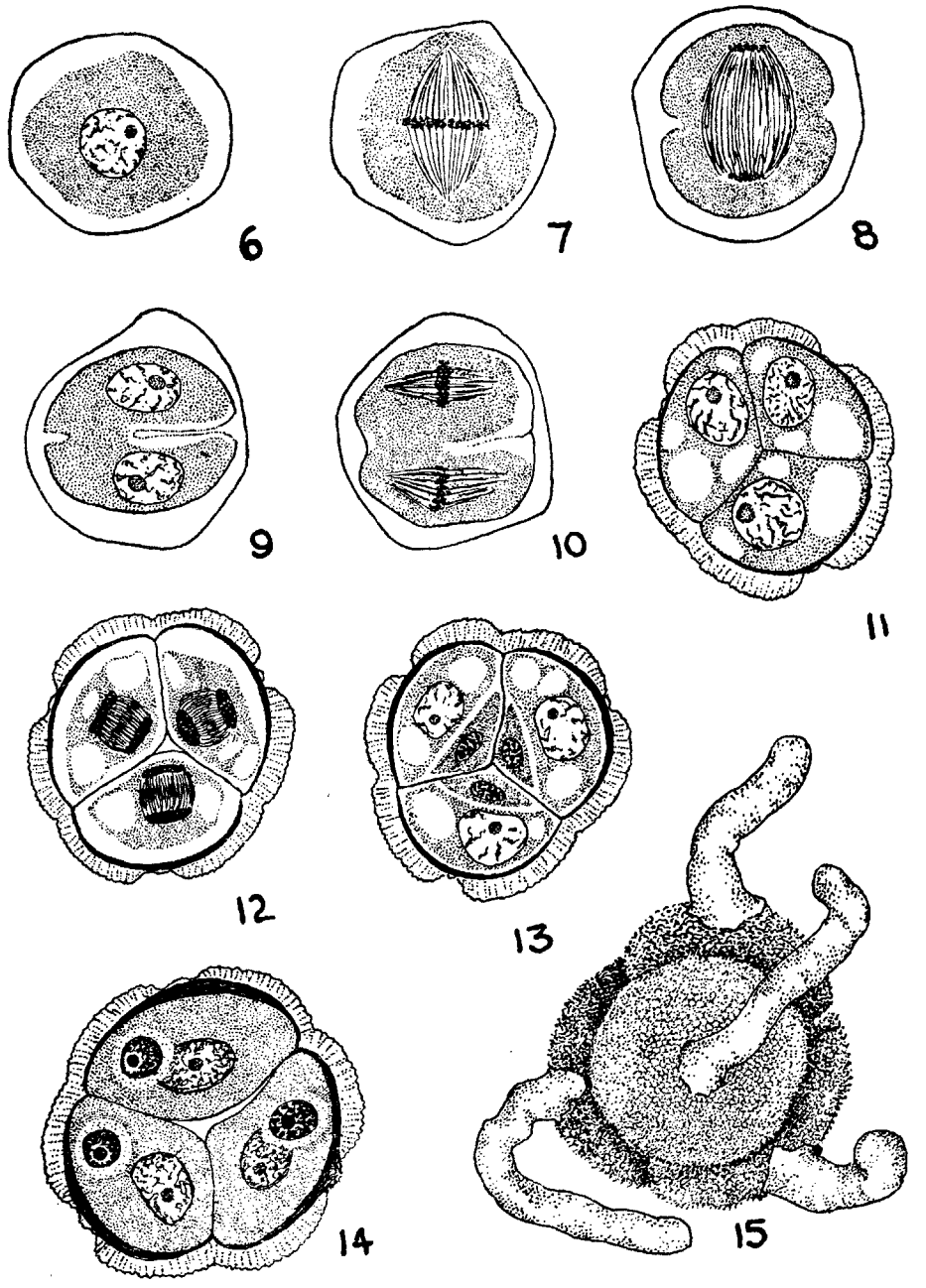
At the time of quadruplication of the microspores, the tapetum embarks on its active phase. The cells distend, the vacuoles become larger, the texture of the cytoplasm coarser, and the nuclei divide into two (Fig. 2). When the microspores complete the cutting off of the generative and vegetative cells, the once divided tapetal nuclei exhibit stages in reunion (Fig. 3). The maximum activity of the nutritive layer appears to diminish gradually after complete fusion of the nuclei; the cell wall together with the cytoplasm becomes very coarsely granular, the nuclei degenerate, and at the time of differentiation of the spore walls, the entire tapetum disintegrates *in situ* (Fig. 4).

The microspore mother cell rounds off by losing its polygonal contour and considerably enlarges before commencing the reduction divisions (Fig. 6). Organisation of a metaphase spindle (Fig. 7) is accomplished with the usual succession of changes accompanying normal meiosis, and while the daughter chromosomal groups



FIGS. 1–5. Fig. 1. Transection of a young anther loculus showing epidermis, three middle layers, tapetum, and microspore mother cells, ×200. Fig. 2. Same, at a later stage, showing nuclear division in the tapetal cells, ×200. Fig. 3. Still older stage, showing the fusion of the nuclei in the tapetal cells, and the beginnings of endothecial thickenings in the two subepidermal layers, ×200 Fig. 4. Same, at maturity, showing the disorganisation of tapetum *in situ*, and a well developed two-layered endothecium, ×200. Fig. 5. Transection of an anther at anthesis, showing the extent of endothecial tissue (semi-diagrammatic), × 60.

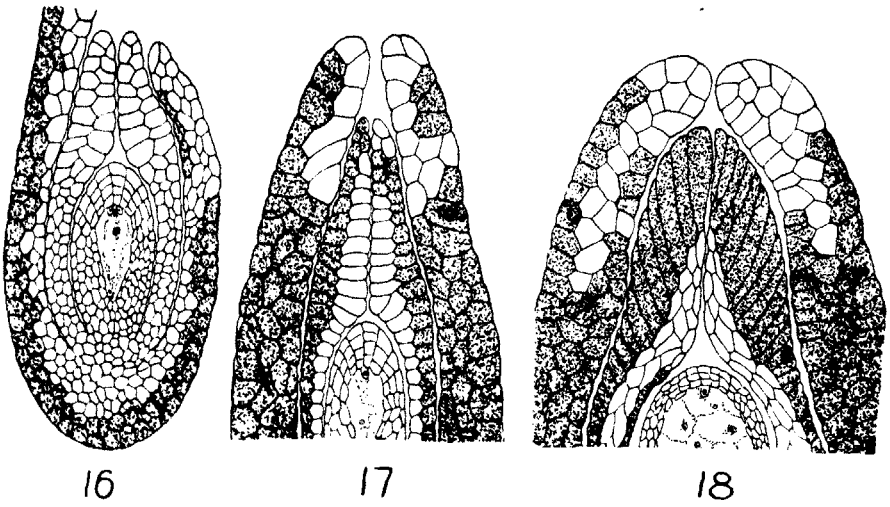
are passing through the later stages of anaphase, the cytoplasm shows the beginnings of an equatorial constriction (Fig. 8). At the time of preparation of the daughter nuclei (Fig. 9) for the ensuing division II (Fig. 10), the constriction advances centripetally in the form of a furrow and at times an incipient cell plate



FIGS. 6–15. Stages in development of pollen grain. Fig. 6. Microspore mother cell. Fig. 7. Metaphase of division I. Fig. 8. Late anaphase of division I. Fig. 9. After telophase I, note commencement of transverse furrow. Fig. 10. Metaphase of division II. Fig. 11. Tetrad of microspores. Fig. 12. Division of microspore. Fig. 13. Formation of generative and vegetative cells. Fig. 14. Tetrad of microspores at the time of shedding. Fig. 15. Germinating tetrad teased out from stigmatic surface. All figures, ×900.

may also be deposited partially (Fig. 10). However, total quadripartitioning is achieved more or less simultaneously only after the completion of division II (Fig. 11). In the greatest majority of cases the microspores are arranged tetrahedrally, a tetragonal configuration being exceptional (Fig. 10).

The uninucleate microspore, soon after its formation, exhibits a few large vacuoles in the cytoplasm (Fig. 11). The nucleus moves towards the proximal pole (in relation to the tetrad) and divides (Fig. 12). The orientation of the spindle is consistently such that the generative cell is always cut off towards the inner, proximal side (Fig. 13). The larger vegetative cell with its nucleus of similar magnitude continues to show the presence of a few large vacuoles, while in contrast the smaller generative cell has a non-vacuolate, dense, deeply staining cytoplasm and a nucleus of similar dimension and staining reactions. At a little later stage the generative cell comes to lie within the cytoplasm of the vegetative cell by which time the cytoplasm of the latter has become non-vacuolate and homogeneous, and that of the generative cell, on the other hand, hyaline (Fig. 14). The position occupied by the generative cell is now variable even among the different pollen

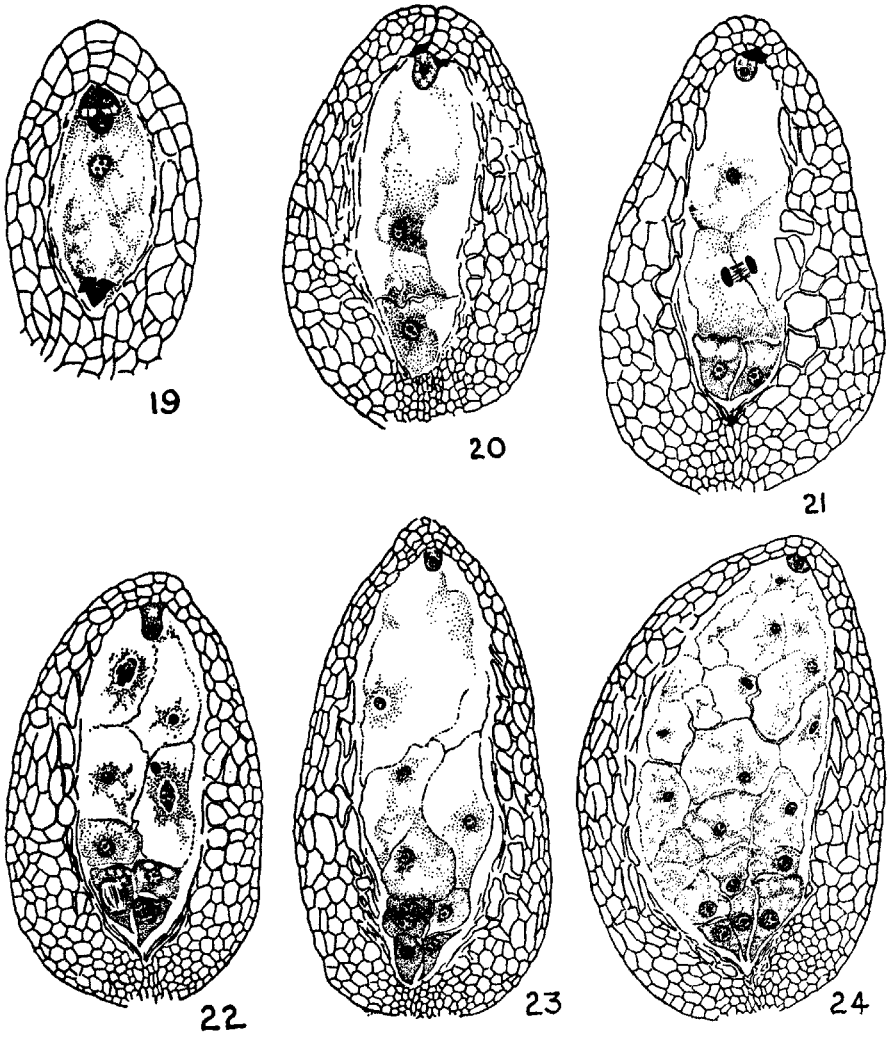


FIGS. 16–18. Fig. 16. Longisection of ovule at the time of fertilisation, ×120. Fig. 17. Micropylar part of same, about the 4-celled stage of endosperm, ×80. Fig. 18. Same, at a slightly later stage, ×60.

grains of the same tetrad. The generative nucleus continues to exhibit a greater avidity for stains while the vegetative nucleus behaves in the reverse direction. The microspores of a tetrad do not separate from one another and germinate as such (Fig. 15) on the stigma.

The property of the microspores to adhere in tetrads affords special facility to determine the exact place of origin and differentiation of the germinal area. Soon after the completion of division II, the spore walls begin to organise. Until the stage shown in Fig. 10, the wall of the mother cell appears as a uniformly thick and highly translucent envelope surrounding the protoplast. Simultaneously with quadruplication through furrowing there is a rapid thinning out of the wall at the central region on the distal face of the microspore. This results in the formation of a shallow pit, which, as seen in a section, appears as a discontinuous region of the spore wall (Fig. 11). This break in the wall later functions as the germ pore (Figs. 12–15). Accompanying these modifications of the external surface of the

spore, there is also accomplished a differentiation of the exine and intine. A major bulk of the thick spore wall towards the exterior appears to become traversed by numerous, highly tenuous capillaries so as to present a radially striated pattern as seen in sections. This part of the wall functions as the exine, and due to



FIGS. 19–24. Longisections of ovules (integuments not shown) illustrating the early stages in the development of endosperm. Fig. 19. Embryo sac at the time of fertilisation, ×220. Fig. 20. Same, after first division of the primary endosperm nucleus, ×220. Fig. 21. Same, at about the 5-celled stage of endosperm, ×220. Figs. 22–24. Later stages, ×120.

this structrual detail, presents the characteristic minutely granular-reticulate sculpturing in the mature pollen grain (Fig. 15). In the initial stages the intine becomes discernible as a thin membrane immediately underlying the germ pore, and gradually extends all over the distal sphere of the cell. However, it presents

maximum thickness at the region of the germ pore and steadily thins out towards the sides. A differentiation *pari passu* results in the slight protrusion of the intine through the germ pore (Fig. 14) long before the dehiscence of the anther.

The germination of the pollen grain takes place in nature only after its transference on to stigma. The intine pushes itself out through the germ pore to form the pollen tube. The emergence and early growth of pollen tubes from a tetrad are synchronous (Fig. 15).

Ovule.—At anthesis, the ovule shows a completely organised, fertilisable embryo sac, the micropylar region of which is over-topped with four or five layers of parietal tissue. The outer integument at this stage is made up of three layers of cells towards the micropylar half and irregularly of four layers towards the base. The cells of the outer two layers show conspicuous brownish infiltration indicated by stippling in Fig. 16. The inner integument is two layered towards the micropyle and gradually becomes three layered towards the chalaza. Also, both the cell layers in the micropylar part of this integument are conspicuously distended. After fertilisation, brownish contents rapidly appear not only in almost all cells of the outer integument, but also in the outer cell layer of the inner integument (Fig. 17). In still later stages, when the embryo sac cavity becomes obliterated due to the filling in of large endosperm cells, the cells of the outer layer of the inner integument in the micropylar region become excessively elongate in a radial direction, whereas those of the inner layer divide to give rise to two or three more layers (Fig. 18).

Endosperm.—The mature embryo sac is normal both in its contained number of nuclei and in their disposition. The polar nuclei fuse before fertilization. The antipodals organise into cells (Fig. 19). Both the synergids appear to undergo degeneration soon after fertilisation. The primary endosperm nucleus may be assumed to migrate slightly towards the chalazal end as can be judged by the disposition of the wall ensuing the first division (Fig. 20). As a result, the embryo sac cavity becomes halved into a larger micropylar chamber and a smaller chalazal chamber. The latter invariably shows a denser and darkly staining accumulation of cytoplasm and this feature seems to be carried through considerably to its derivatives, perhaps even up to slightly later stages than is represented in Fig. 24, as may be evidenced by a clear gradient in the intensity of staining of the endosperm tissue from the chalazal pole towards the embryo (Figs. 21–24). There is also a graded difference in the nuclear size of the respective cells (Figs. 23, 24), although this feature may not be manifest at the two-celled stage of the endosperm (Fig. 20). Subsequent divisions of both the larger micropylar and the smaller chalazal chambers obviously proceed at the same rate, so that cells of corresponding sizes are derived. However, the sizes soon intergrade in the border zone. The oldest stage of endosperm that was observed is illustrated in Fig. 24, and until this time the zygote has not divided.

Discussion.

Neither detailed comparison nor comprehensive discussion is possible at present with regard to the embryological characters of *Zygogynum*. Of the six known genera,—*Drimys*, *Bubbia*, *Belliolum*, *Exospermum*, *Pseudowintera*, and *Zygogynum* —of the Winteraceae,[1] only *Drimys* has been studied from the point of view of embryology; the information available is, however, too meagre to allow dependable conclusions.

The casual remarks of Willie (1886) on the properties of the spore walls of *Drimys* pollen grain, taken by itself, are of very little significance for the present

[1] See Smith, A. C., in Jour. Arnold Arb. 24: 1–33, 119–164. 1943.

purpose. The observations of Bhagavathi Kutti Amma (1938), although accompanied by a series of illustrations, fail to provide an accurate picture of the sequence of events, and several points therein are badly in need of critical reinvestigation. It is not clear from her account whether it is the tapetal *cells* or *nuclei* that undergo division; nor has it been ascertained whether the once divided nuclei reunite. Furthermore, she writes that, 'In the metaphase stage of the pollen mother cell, the tapetal cells lose their walls completely and persist as a mass of cytoplasm with one or two nuclei in each. They encroach in between the pollen mother cells.... When tetrads are formed, the tapetal cytoplasts completely fuse together and become the periplasm. The nuclei become amoeboid and lie free in the periplasm'. The sequence of events obviously is indicative of the organisation of tapetal periplasmodium. If this observation is correct, *Zygogynum* deviates from *Drimys* in exhibiting a typically secretory type of tapetum.

Although Bhagavathi Kutti Amma does not describe the details involved during the first division of the microspore in the text, she has provided illustrations (see her figure 19, Pl. III, figures 10, 11, Pl. IV) which clearly demonstrate that the generative cell is cut off towards the proximal pole of the pollen grain and that the germ pore is organised on the distal face. Both these features are also shared by *Zygogynum*. The constancy in the locus of the generative cell (towards the proximal pole) in the Winteraceae provides a good point of contrast with that (towards the distal pole) in the Magnoliaceae *sensu stricto*[1] (unpublished observations of the author on species of *Michelia*, of *Magnolia*, of *Tauluma*, and *Liriodendron*) and its close relative, the Degeneriaceae (Swamy, 1949).

Strasburger (1905) reports a nuclear type of endosperm development in *Drimys Winteri*. If this is correct, both nuclear and cellular (*Zygogynum*) types occur within the family.

The conspicuous radial elongation of the outer layer of cells of the inner integument in the region of the micropyle during post-fertilisation development of *Zygogynum* appears to be a constant feature also in *Drimys* and *Bubbia* (unpublished observations of the author). Such a differentiation is absent in the corresponding tissue of the Magnoliaceae. A much more significant point of difference lies in the structure of the seed coat. As could be ascertained from herbarium specimens of *Zygogynum* and *Bubbia*, and also from the generality of observations of Miers (1858) on *Drimys*, the seed structure of the winteraceous genera appears to be rather uniform. The cells of the outermost layer of the outer integument develop dark sclerotic walls and alone persist in the mature seed as a black, shiny crest; all other cell layers of the outer and inner integuments become crushed. In marked contrast, in the seeds of the Magnoliaceae and Degeneriaceae, the outer integument becomes characteristically differentiated into outer fleshy and inner stony regions, the former becoming pulpy and juicy at maturity.

In the prevailing well-known systems of angiosperm classification it has been the custom to assign *Drimys* and its presumed allies to the Magnoliaceae either as members of a special tribe or a subfamily, implying a rather close natural relationship. The recent investigations of Prof. I. W. Bailey and his collaborators have clearly demonstrated—at least as far as the vegetative characters are concerned—that the trends of phylogenetic specialisation have progressed along divergent lines among the different units of the Magnoliaceae, and that 'to include such morphologically dissimilar elements as the Winteraceae, *Illicium*, the Schisandraceæ, and *Tetracentron* in the Magnoliaceae broadens the concept of this family even beyond the limits of a natural suborder'. With particular reference to the Winteraceae, Bailey and Nast (1945) conclude that although the genera of the family are of general ranalian affinities, 'they do not appear to be closely related to any specific surviving family of the ranalian complex' (see also Bailey and Smith, 1942). The

[1] See Dandy, J. E., in Kew Bull. 1927, pp. 257–264.

few salient points of contrast in the embryological characters of the Winteraceae on the one hand and those of the Magnoliaceae and Degeneriaceae on the other, as presented above, appear to indicate two very remotely related trends of evolutionary modifications, thereby justifying the dissociation of the Winteraceae from the magnoliaceous alliance.

Summary.

The precocious appearance of cytoplasmic cleavage towards the end of the first reduction division in the microspore mother cell, and the differentiation of endodermal thickenings in more than one layer of cells of the anther wall are points of interest in *Zygogynum Bailloni*.

The tapetum is of the secretory type, the cells becoming binucleate and finally again uninucleate due to fusion.

The generative cell is cut off towards the proximal pole and the germ pore differentiates on the distal face of the grain.

The ovule is bitegumentary and crassinucellate. The mature embryo sac is eight nucleate with typical organisation. The endosperm is *ab initio* cellular. The seed coat consists of the modified outermost cell layer of the outer integument. The outermost cell layer of the inner integument in the neighbourhood of the micropyle undergoes pronounced radial elongation during early post-fertilisation development.

The embryological characters of the Winteraceae, on the one hand, and of the Magnoliaceae and Degeneriaceae, on the other, appear to indicate two very remotely related trends of evolutionary modifications, thereby justifying the dissociation of the Winteraceae from the magnoliaceous alliance.

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