SOME OBSERVATIONS ON THE EMBRYOLOGY OF *DECAISNEA INSIGNIS* HOOK. ET THOMS [1]

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(Communicated by Prof. P. Maheshwari, F.N.I.)

(*Received July 31 ; read October 4, 1952.*)

INTRODUCTION.

As suggested in a previous paper (Swamy and Bailey, 1949), the families of ranalian affinities as broadly conceived by Engler and Prantl may be regrouped to advantage under at least two major categories: (1) having monocolpate pollen or phylogenetically modified types of such pollen, and characteristic secretory cells ('ethereal oil cells'), and (2) having tricolpate pollen or types derived therefrom, and no 'ethereal oil cells'. In the latter group belong, among others, Ranunculaceae, Berberidaceae, Lardizabalaceae, and Menispermaceae. These four families are generally assumed to exhibit closer relationship among themselves than with other families of the category. In view of the recent results that are being obtained by comparative morphological and anatomical studies on the ranalian families, it appears very desirable to supplement data from embryological investigations on these and other families of the Ranales.

The only information available on the embryological characters of the Lardizabalaceae are those provided by the casual accounts of Vesque (1879) on *Holboellia latifolia* and of Vesler (1913) on *Akebia quinata*. Both the authors report the formation of a parietal cell in the ovules of the respective species investigated by them and Vesler's slightly extended observations, although inadequate, concern the development of the embryo sac and of pollen grain.

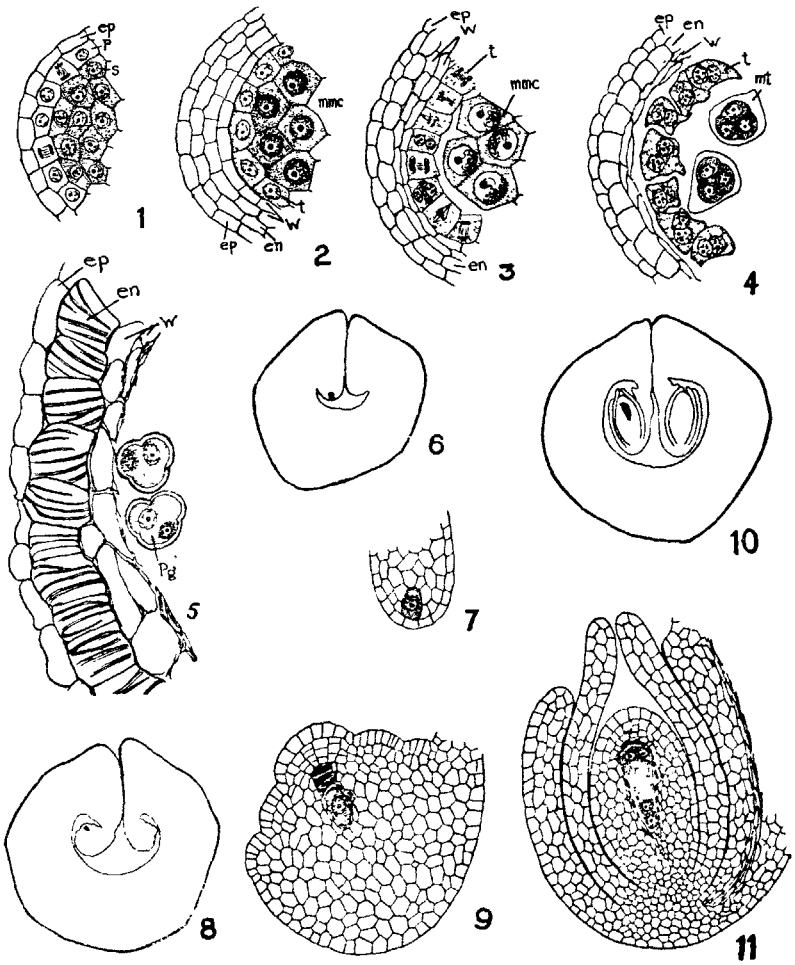
OBSERVATIONS.

Development of the pollen grain.—Fig. 1 represents an early stage in the development of an anther. The outermost layer of cells is the epidermis (*ep*) and the second is obviously a derivative of the archesporium and functions as the primary parietal layer (*p*). The cells towards the interior are arranged in a compact mass and constitute the sporogenous tissue (*s*). The parietal row of cells gives rise to four layers: the outermost differentiates into endodermis (Figs. 2–5, *en*); the innermost functions as tapetum (Figs. 2–4, *t*); the middle two layers constitute the wall tissue (Figs. 2–5, *w*). When the microspore mother cells are passing through mid-prophasic development, the tapetal cells become binucleate (Fig. 3, *t*) and reach maximum activity when microspore tetrads are organized (Fig. 4, *t*). At this stage the individual cells of the tapetum exhibit a less angular contour, a coarsely granular and vacuolate cytoplasm, and prominent nuclei. Their activity appears to slow down gradually and the structure degenerates by the time the microspores are organized. In the mature anther the epidermis persists as also one or irregularly two layers of wall tissue in a somewhat crushed condition in addition to the well developed endothecium (Fig. 5); however, the cells are devoid of protoplasts. The sequence of stages in the development of pollen grains does not present

[1] The material was obtained in 1949 from a plant under cultivation in the Arnold Arboretum, Massachusetts, U.S.A.

any unusual features. Thus, the meiotic divisions take place in a simultaneous manner; the disposition of microspores of a tetrad is tetrahedral; the pollen grains are two-celled at the shedding stage (Fig. 5).

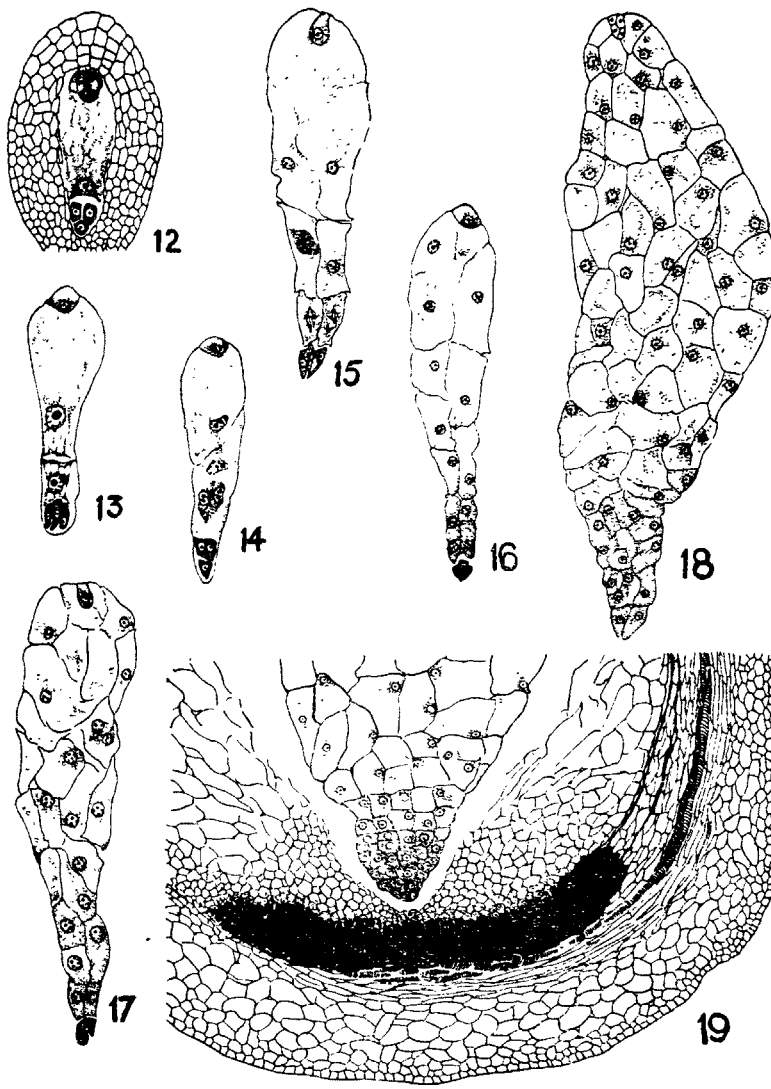
Development of the embryo sac.—The ovules are arranged in two longitudinal series, considerable distance away from the actual margins of the megasporophyll



Figs. 1–11. Figs. 1–5. Transections of a part of anther locule illustrating successive stages in the development of anther wall and pollen grain, ×50. *en*—endodermis; *ep*—epidermis; *mmc*—microspore mother cells; *mt*—microspore tetrad; *p*—parietal layer; *pg*—pollen grain; *s*—sporogenous tissue; *t*—tapetum; *w*—wall layers. Fig. 6. Outline of transection of carpel, ×12·5. Fig. 7. Ovular primordium from Fig. 6 enlarged to show the archesporial cell, ×32·5. Fig. 8. Outline of transection of carpel slightly older than in Fig. 6, ×12·5. Fig. 9. Ovule in Fig. 8 enlarged to show the origin of integuments and linear tetrad of megaspores, the three micropylar ones degenerating, ×32·5. Fig. 10. Outline of transection of carpel at the time of pollination, ×12·5. Fig. 11. Longisection of ovule from Fig. 10, showing the organization of micropyle and four-nucleate embryo sac, ×32·5.

(Fig. 10; see also Bailey and Swamy, 1951). The ovule primordium arises as a tiny protuberance directed towards the dorsal side of the carpel and even at so early a stage as this, the single hypodermal archesporial cell stands fully differentiated (Figs. 6, 7). Subsequent growth results in a 180° curvature of the longitudinal axis of the nucellus and integuments so that about the four-nucleate

stage of the embryo sac the ovule attains typically anatropous position (Figs. 10, 11). The two integuments are initiated simultaneously with sporogenesis and their pre-fertilization development is completed when the female gametophyte is tetra-nucleate (Fig. 11). The inner integument is uniformly of three cell layers thickness and the outer, at this stage, consists of four cell layers.



FIGS. 12–19. Fig. 12. Longisection of nucellus prior to fertilization, showing mature embryo sac, ×32·5. Fig. 13. Two-celled endosperm, ×32·5. Figs. 14–18. Representative stages in the subsequent development of endosperm, Figs. 14–16, ×32·5; Fig. 17, ×22·5; Fig. 18, ×17·5. Fig. 19. Longisection of chalazal part of ovule showing the position of darkly staining pad of cells and its relation to other tissues of ovule, ×12·5.

The archesporial cell divides by a periclinal wall to form perietal and sporogenous cells. The former by successive divisions builds up a parietal tissue of three or four layers, and the latter functions as the megaspore mother cell. A

typical linear tetrad of megaspores is formed as a result of meiotic divisions; the chalazal megaspore matures into an eight-nucleate embryo sac (Figs. 9, 11, 12). The antipodal nuclei organize into cells, and persist without any morphological change during post-fertilization development until as late a stage as in Fig. 17. The polar nuclei fuse before fertilization and the resulting nucleus occupies a constant position nearer to the antipodal group (Fig. 12).

Endosperm.—The triple-fused primary endosperm nucleus divides *in situ* and promptly a cell membrane is organized between the daughter nuclei. This results in the partitioning of the embryo sac into a larger micropylar and a smaller chalazal chambers (Fig. 13). Both the compartments appear to have an equal share in the building up of endosperm. Nuclear divisions in earlier stages proceed more or less simultaneously in the two chambers and walls formed during divisions intersect one antoher in all planes so that a distinction between the derivatives of the two chambers soon disappears, although the cells at the micropylar end are considerably larger than those at the opposite pole (Figs. 14–16). However, during following stages, the cells at the chalazal end accumulate densely staining protoplasts and begin to multiply at a relatively faster rate than those towards the opposite end (Figs. 16–18), and this activity appears to continue steadily even after cells of more or less uniform size and appearance fill the remainder of the embryo sac (Figs. 18, 19).

During post-fertilization development, the inner integument soon becomes crushed. The outer integument increases in thickness owing to divisions in its cells and in the oldest stage available to me (as in Fig. 19), contains five or six cell layers. Hand in hand with the development of endosperm, a pad of tissue lying between the base of integuments lose their cell contents and the cavities become filled with darkly staining infiltrations (Fig. 19).

Summary.

Decaisnea insignis agrees with *Holboellia latifolia* and *Akebia quinata* in the possession of krassinucellate and bitegumentary ovules and in the formation of a parietal cell which in turn produces three or four layers of similar tissue. As in *Akebia quinata*, Polygonum type of development characterizes the female gametophyte of *Decaisnea*. Organization of a binucleate and secretory type of tapetum, simultaneous method of divisions in the microspore mother cell, development of a single layered endothecium, and two-celled shedding condition of the pollen, are characters shared both by *Akebia* and *Decaisnea*.

The location of the secondary embryo sac nucleus in *Decaisnea* is characteristically nearer to the antipodal cells than to other component cells of the embryo sac, a situation that appears to be paralleled in *Akebia* as can be judged by Vesler's illustrations. In *Decaisnea*, the division of the primary endosperm nucleus is followed by the formation of a wall, thereby halving the embryo sac into a larger micropylar and a smaller chalazal chambers. Both the chambers contribute towards the building up of endosperm. Towards later stages the rate of divisions of endosperm cells situated nearest to the chalazal end appear to become accelerated. Four to six cell layers inclosed by the base of integuments differentiate into an opaque, darkly staining pad of tissue during post-fertilization development.

Acknowledgements.

I am deeply grateful to the National Institute of Sciences of India, Arnold Arboretum of Harvard University, U.S.A., and to the Madras University for having extended to me opportunities to study this and other problems.

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Issued April 29, 1953