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# The Development of the Embryo Sac in Ranunculus Glaberrimus Hook

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114

# THE DEVELOPMENT OF THE EMBRYO SAC
# IN *RANUNCULUS GLABERRIMUS HOOK*

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A Thesis
Presented to
the Graduate Faculty
Central Washington State College

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In Partial Fulfillment
of the Requirements for the Degree
Master of Science

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by
Chung-ping Chin
August, 1968

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APPROVED FOR THE GRADUATE FACULTY

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M. W. Mayberry

# ACKNOWLEDGMENTS

I wish to thank, at this time, Dr. William W. Barker for his guidance and assistance during my times on this campus.

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# TABLE OF CONTENTS

# CHAPTER I

# INTRODUCTION

According to a generally held conception, the female gametophyte among angiosperms is to be regarded as a structure which has passed through considerable evolutionary change and is now reduced to a few cells. It is considered to be homologous with the independent gametophytic generation of lower Tracheophytes, but among angiosperms, has become essentially as organ of the sporophyte. It is the culmination of an evolutionary trend toward increase in size and complexity of the sporophyte along with a reduction of the gametophyte. In the bryophytes the gametophyte is the dominant independent part of the plant, the sporophyte, although multicellular, is dependent upon and nourished by the gametophyte. Among ferns another step in the progressive evolution of the sporophyte is seen. The gametophyte is independent but small and inconspicuous; whereas the sporophyte is large and structurally specialized and, early in its ontogeny, becomes independent of the gametopyte. The culmination of the progression is seen in flowering plants. In them the gametophyte is reduced to a few nuclear divisions in the pollen grain and in the embryo sac, the gametophyte is completely dependent upon the sporophyte for its development.

It is generally observed that structures which have experienced considerable evolutionary change and reduction also show a high degree of variability. The embryo sac of angiosperms is no exception. Maheshwari (1948) has developed a classification of the various types and subtypes of embryo sac development in the angiosperms. His three major categories are the monosporic type, bisporic type, and tetrasporic type. In the monosporic type megasporogenesis results in four, well-defined megaspore, only one of which gives rise to the embryo sac; the definitive feature of the bisporic type arises from the abortion of one of the two dyad cells produced after the first meiotic division of the megasporocyte, the nucleus of the surviving dyad cell (commonly the lower chalazal dyad cell) divides to form two haploid nuclei which are megaspore nuclei; the tetrasporic type of embryo sac development is remarkable because of the complete elimination of wall formation during meiosis, and the participation of all four megaspore nuclei in the formation of the embryo sac. A review of the literature reveals that two types of embryo sac development have been found in the family Raunuculaceae. *Caltha palustris* has shown by Kapil and Jalan (1960) to possess a female gametophyte which follows the monosporic (*polygonum* type) of development. On the other hand, Bhandari (1962) identified a bisporic (*Allium* type) development of the female gametophyte in *Adonis annua*. The purpose of the present research is to try to identify the type of embryo sac development followed by *Ranunculus glaberrimus Hook*.

Ranunculus glaberrimus Hook, the sagebrush buttercup, is a terrestrial herbaceous member of Ranunculaceae. It ranges in height from 4 to 8 centimeters, with large and numerous roots. The stems are decumbent and the leaves are round or ovate and usually 3-5 lobed at the apex. The flowers of Ranunculus glaberrimus Hook arise singly or in groups of 2-3 in axils of cauline leaves. They are complete, actinomorphic, and bisexual. The androecium consists of numerous stamens, and the gynoecium contains numerous free, superior pistils. Each flower possesses five broadly-ovate, bright-yellow petals. The früits are achenes.

According to Benson (1936), this species occupies a range reaching from British Columbia to California and western Colorado, and is one of the first flowers of spring in the Columbia River Basin and the Northern Rocky Mountains.

Leppik (1964) points out that most taxonomists such as Bessy, Hallier, Hutchinson, Tippo, Cronquist, Eichler, Eames, and Thorne today consider the family Ranunculaceae to be a natural taxon with well-preserved primitive characteristics. Phylogenetically it is probably among the lower most group of dicots. The primitive characteristics of Ranunculus glaberrimus Hook are: they possess a perianth, are bisexual and actinomorphic, have numerous carpels and stamens and the parts of the flower are distinct from one another.

# CHAPTER II

# MATERIAL AND METHOD

Flowers of Ranunculus glaberrimus Hook were collected at several locations around Ellensburg, Washington; specifically Colockum Pass, Dry Creek, Saddle Mountain, and also near Leavenworth during the months of March, April and May of 1967 and 1968.

To prepare the flowers for sectioning, sepals, petals and stamens were removed, leaving only the numerous pistils attached to the receptacle. The pistils and receptacles were then fixed in Randolph's Modified Navashin Fixative. The fixative was prepared as two separate mixtures, A and B, which were then mixed 1 : 1 just prior to use. Solution A consists of one gram Chromic acid, 7 cc. Glacial acetic and 92 cc. distilled water (based on 100 units); solution B was made up of 30 cc. Neutral formalin and 70 cc. distilled water (based on 100 units). Materials were fixed for at least 24 hours. Prior to fixation the flowers were separated into four age classes: A, B, C, and D. Those flowers which were in fullest bloom with the petals starting to fall off and pollen sacs broken open were classified as class A. Flowers which were quite open, with petals showing a deep yellow color but not yet dropping and pollen sac not yet open, were classified as Class B. Those flowers which were just starting to open with pistils and stamens still a light green color and with small, light-yellow petals, were classified as class C. Flowers still in bud were classified as class D. Stages pertinent to the study of embryo sac development were found mainly in the "C" and "D" classes.

Following fixation, materials were treated in a Tertiary-Butyl-Alcohol (TBA) series. Tertiary butyl alcohol was used in accordance with the prinicpals of dehydration. Materials were passed through the following five steps:

| No. of step | 95% Ethyl alcohol | Absolute Ethyl alcohol | TBA | Water |
|---|---|---|---|---|
| 1 | 50 | 0 | 20 | 30 |
| 2 | 50 | 0 | 35 | 15 |
| 3 | 45 | 0 | 55 | 0 |
| 4 | 0 | 25 | 75 | 0 |
| 5 | Equal volume of TBA and paraffin oil | | | |

Each step required at least 24 hours. Following dehydration the material was imbedded in paraffin as recommended by Johansen (1940). The ovaries, still attached to the receptacle, were sectioned at 10-20 microns in a medium longitudinal plane. The sections were stained with Gentian-Violet-Iodine (method recommended by Smith). The procedures followed the next 12 steps:

1. Run down to 70% ethyl alcohol.
2. Mordant 10 to 20 minutes in an IKI solution (one gram I, 1 gram KI, in 100 ml. 80% alcohol).
3. Rinse in water.
4. Stain in boiled and filtered crystal Gentian Violet (1% aquous) for 20 minutes.
5. Rinse in water.

6. Rinse in IKI solution for a few seconds.
7. Rinse in 95% alcohol.
8. Flood slide quickly with a saturated solution of picric acid in absolute ethyl alcohol.
9. Wash slide immediately with absolute ethyl alcohol for only a few seconds.
10. Flood slide with clove oil to differentiate.
11. Place in xylene to remove clove oil.
12. Mount in balsam.

Photomicrographs were taken through a Leitz Ortholux microscope and were recorded on Kodak panatomic-x film.

# CHAPTER III

## OBSERVATION

In Ranunculus glaberrimus Hook the ovary is strickly superior and contains a single anatropous unitegminal ovule, (fig. 6). Foster and Gifford (1959) point out that the integument of the ovule still remains a problem. The evolutionary significance of unitegmic (one integument) and bitegmic (two integuments) ovule is by no means clear. Rarely the ovules remain campylotropous.

In the ovule primordium the nucellus develops first. The growth of the nucellus then slows as the integument begins to develop and the archesporium differentiates (fig. 1). Archesporial cells characteristically have dense cytoplasm and enlarged nuclei. The archesporium is multicelled but only one cell becomes a megasporocyte. (fig. 2, 3, 9).

The parietal cells of this species are well developed. The megasporocyte is further buried in the nucellus by periclinal and anticlinal divisions of the parietal cell derivatives (fig. 4). Therefore, the ovule is termed a crassinucellate type. However, the development of the embryo sac is at the expense of the parietal cells, hence at maturity the embryo sac lies next to the nucellus epidermis. Foster and Gifford also mentioned that the phylogenetic significance of parietal cells is problematical, although it has been argued that there is a strong tendency toward the elimination of such cells in angiosperm evolution.

The megasporocyte undergoes meiosis to form four megaspore nuclei, which show varying positions with respect to one another, but eventually arrange themselves in two pairs at the opposite ends of the embryo sac (fig. 5, 7, 8, 10, 11). A third nuclear mitotic division produces eight nuclei which become organized to form an eight-nucleate embryo sac (fig. 12, 13). An egg and two synergids form at the micropylar end, while three antipodals organize at the chalazal region. The two polar nuclei are prominent in the middle of the embryo sac (fig. 14).

Since all four megaspores are involved in the formation of embryo sac in Ranunculus glaberrimus Hook, the embryo sac is classified as a tetrasporic type.

Fig. 1-8. Fig. 2, 3, 4, 5, x 400; Fig. 1, 6, x 250; Fig. 7, 8, x 400. Fig. 1, Ovule as a primordia. See the integumentary primordia arise from the nucellar mass. Fig. 2, Ovule at archesporial cell stage; Fig. 3, Ovule at megasporocyte stage; note the enlarged nucleus. Fig. 4, Ovule show crassinucellate type; Fig. 6, two nucleate megagametophyte stage. Note the well defined single integument. Fig. 7, 8, Ovule at four-nucleate megagametophyte stage; showing varying in position.


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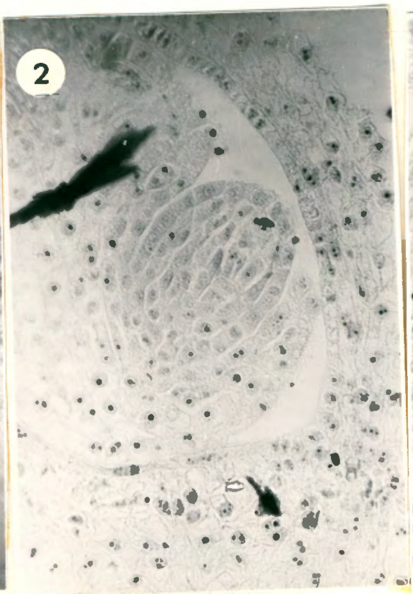
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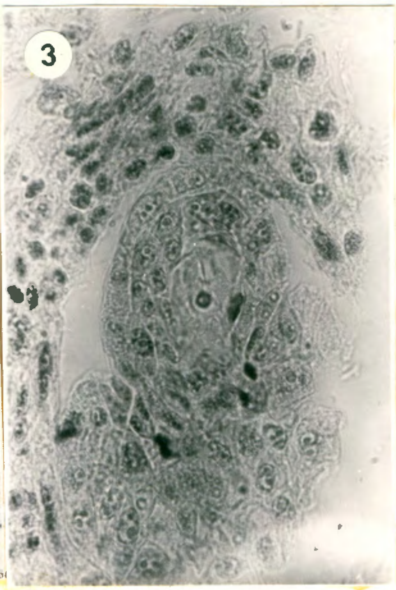
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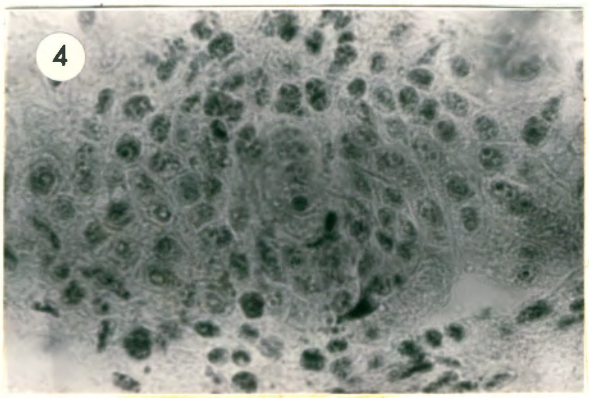
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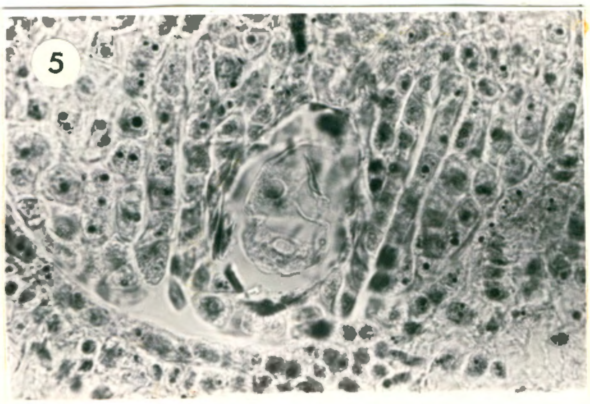
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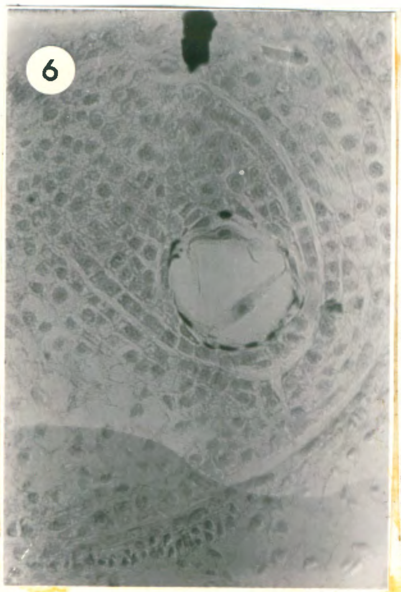
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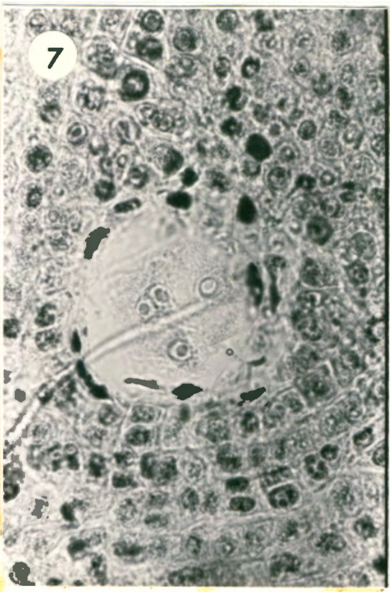
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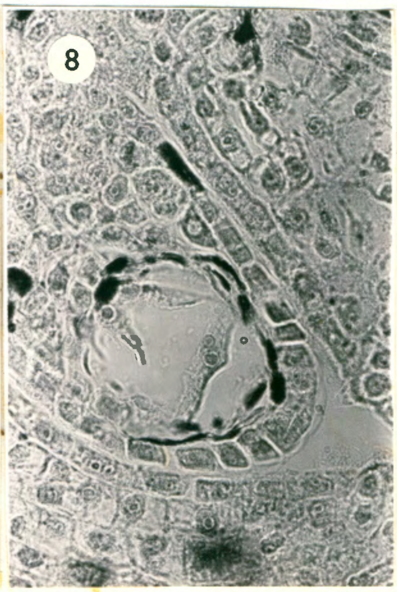
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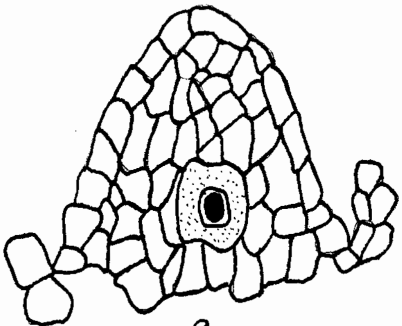
Fig. 9, Ovule at Megasporocyte stage. Fig. 10, Ovule with two nucleate megagametophyte. Fig. 11, Ovule with four-nucleate megagametophyte. Fig. 12, Ovule at eight-nucleate megagametophyte stage. Fig. 13, Mature eight-nucleate megagametophyte egg apparatus at the micropylar end. Fig. 14, Spatial relationship of cells and nuclei of the mature megagametophyte to the mature ovule.

a, antipodals; pn, polar nuclei, ea, egg apparatus; in, integument.



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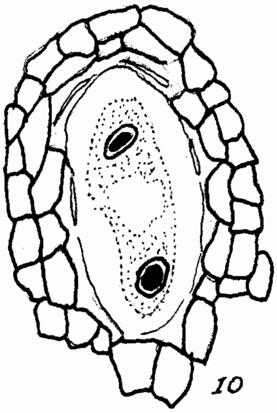

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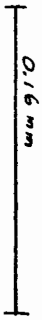

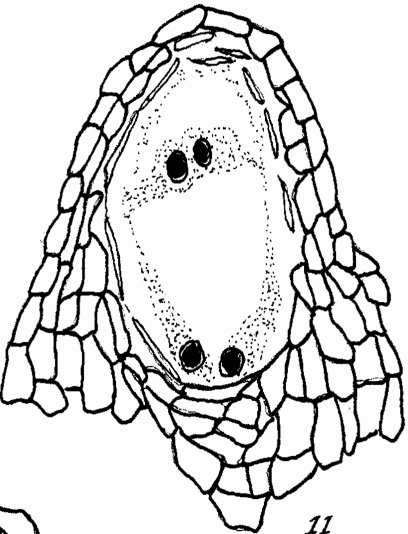

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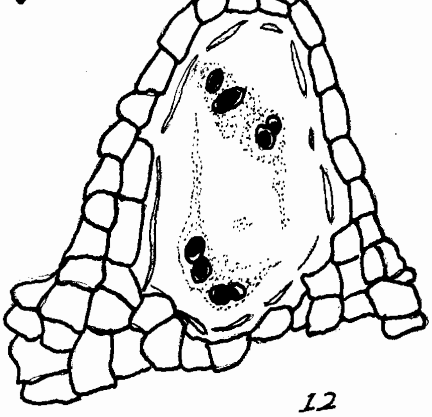

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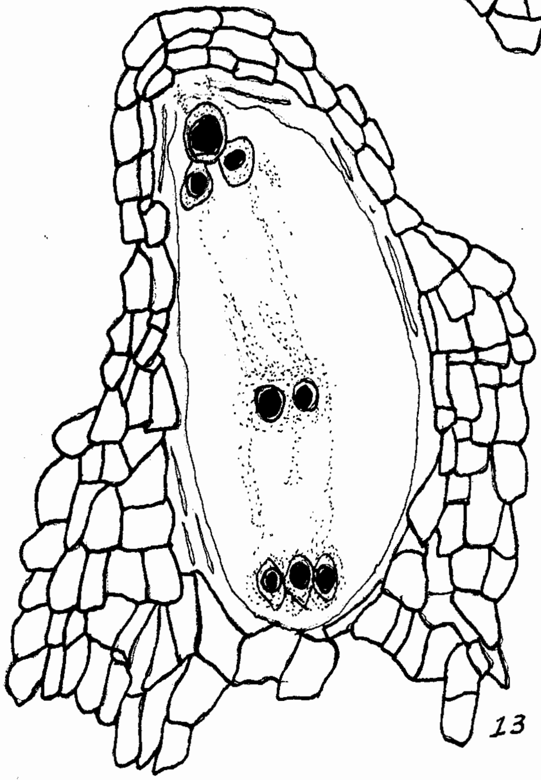

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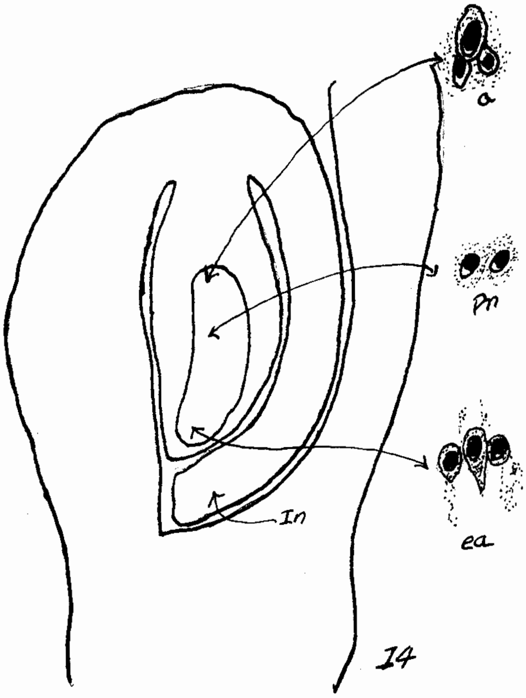

14

# CHAPTER IV

# DISCUSSION

A comparative study of embryological and morphological characters of the ovule in the family Ranunculaceae indicates that considerable variation exists within this family.

Mottier (1895) recorded that in the family Ranunculaceae, except in Aquiegia canadenis where a parietal cell is occassionally cut off, the archesporial cell directly develops into the embryo sac. Dahlgren (1927) seems to rule out any possibility of a parietal cell being cut off in this family. A perusal of the literature, however, reveals that the situation is somewhat different. Dahlgren himself had listed five species Clematis vitalba, Helleborus foetidus, Helleborus spp., Thalictrum purpurascens, and Paeonia spp., where a parietal cell had been reported. Parietal cells are also found in Caltha palustris L. as reported by Kapil and Jalan (1961). In the present study it was found that the parietal cells of Ranunculus glaberrimus Hook are well formed.

Mottier (1895) described the general development of the embryo sac of Caltha palustris L. He noted that "in the ovule the upper (micropylar) dyad cell failed to divide while the lower (chalazal) dayad cell gave rise to a two nucleate embryo sac and subsequently lead to form a bisporic type." But Kapil and Jalan (1961) reinvestigated Caltha palustris L. and describe the embryo sac development as a monosporic type in which megasporogenesis results in four well defined megaspores, only one of which gave rise to the embryo sac. It is, therefore, possible that there are two types of embryo sac development in Caltha palustris L.

Souèges (1912) described monosporic development of the female gametophyte in Adonis autumnalis and A. aestivalis; Bhandari (1962) identified a bisporic (Allium type) development of the female gametophyte in Adonis annua but a tendency for tetrasporic development is seen in the uppermost one of all four sterile ovules; Bhandari (1965) described monosporic (polygonum type) development of the female gametophyte in Anemone vitifolia but in the sterile ovule it is bisporic or tetrasporic. Jalan (1963) described the embryo sac as monosporic (polygonum type) in Actaea spicata. The female gametophyte of Ranunculus glaberrimus Hook is here described as having a tetrasporic (Adoxa type) development in which all four megaspore nuclei are involved in forming the embryo sac.

Except for the possibility of tetrasporic embryo-sac development in the sterile ovules of Adonis annua and Anemone vitifolia, only two types of embryo sac development have been previously reported in the Ranunculaceae, i.e. monosporic in Adonis aestivalis, A. autumnalis, Anemone vitifolia, Actaea spicata, and Caltha palustris L.; bisporic in Adonis annua and possibly Caltha palustris L. With the discovery of a tetrasporic development in Ranunculus glaberrimus Hook it must now be considered that all three types exist in the Ranunculaceae.

As far as embryo sac development is concerned, it is difficult to determine which type, monosporic, bisporic, or tetrasporic, is the more primitive.

Maheshwari (1948) agreed with Schnarf's view and accepted the monosporic, eight-nucleate embryo sac as the most primitive type. The chief arguments supporting this view are that in the pteridophytes and gymnosperms the female gametophyte is always monosporic and also that the monosporic condition is probably the commonest and most widely distributed condition in angiosperms.

While there is some logic supporting the view that the monosporic condition is primitive, it is by no means definitely established. Maheshwari, for instance, also pointed out that Fagerlind had found a tetrasporic origin for the female gametophyte of Gnetum gnemon ovalifolium. Further, there is no marked tendency for the monosporic type of embryo sac to prevail in families otherwise judged to be primitive. The Ranunculaceae, for instance, is considered to be a relatively primitive family within the angiosperms, and yet within this family are found all three types of embryo sac development. In fact, variation in embryo sac development within a taxon seems to be not at all uncommon. One may find variation within a family, a genus, a species, and as was mentioned earlier, Bhandari even found variation among different ovules of a single ovary.

While it is possible that the monosporic, eight-nucleate type of embryo sac may be the fundamental angiosperm type, the evidence, at present, does not clearly establish it as such, and phylogenetic conclusions based on embryo-sac type must be considered as tenuous. Hjelmquist (1964) suggests that phylogenetic considerations are only one factor influencing embryo sac development and that the variation of embryo sac development within a plant family, a genus, a species, or even in a single evary, is probably caused by the interaction of the genetic disposition of the plant, internal nutritional competition and external environmental factors.

# CHAPTER V

# CONCLUSION

The ovule of *Ranunculus glaberrimus Hook* is untiegminal and anatropous with basal placentation. The parietal cell are well developed and the megasporocyte is buried in the nucellus indicating a crassinucellate type nucellus.

The megasporocyte undergoes meiosis to form four haploid nuclei, not separated by cell walls. After one more mitotic division an eight-nucleate embryo sac is formed. An egg and two synergids are located at the micropylar end, three antipodals are found at the chalazal region, and the two polar nuclei are conspicuous in the middle of the embryo sac.

Since all four megaspore nuclei are involved in the formation of the embryo sac, it is a tetrasporic type embryo sac.

Monosporic (*Caltha palustris L.*, *Adonis autumnalis*, *A. aestivalis*, *Actaea spicata*, *Anemone Vitifolia*) and bisporic (*Adonis annua*, *Caltha palustris L.*) embryo sac development has been previously reported in the *Ranunculaceae*. The present research is the first record of a functional tetrasporic embryo sac in this family.

# CHAPTER IV

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