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Development of Ovule and Embryo Sac in Blotched Tree Peony (*Paeonia rockii*)

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Abstract This paper dealt with the ovule ontogenesis and development, the megasporogenesis and the embryo sac development in *Paeonia rockii* cvs. The ovule, originated from the tri-zonate primordium, was anatropous, bitegmic and crassinucellate. And the nucellus consisted of different cytohistological zones and differentiated by a particular way in which the derivative cells from both parietal and sporogeneous cells were contributed to the nucellar tissue. During the megasporogenesis and before the 4-nuclear embryo sac, the megasporocytes successively entered meiosis or commenced to degenerate. Generally a linear tetrad formed after meiosis and the chalazal megaspore was functional and developed into embryo sac by *Polygonum* type. Multicelled archesporium and multiple megasporocytes in an ovule resulted in 1-3 embryo sacs, which developed to mature within several days after anthesis. Belated mature of embryo sacs supplies a device to guarantee the cross-pollination. The characteristics about ovule and embryo sac of peonies were more primitive and imply the primitive of *Paeoniaceae* in phylogeny.

Key Words: development, differentiation, embryo sac, ovule, tree peony.

Introduction

Paeonia (*Paeoniaceae*) consists of three sections, two herbaceous and one woody ones, and is very attractive to horticulturists for two well-known flowers, herbaceous and tree peonies, as well as botanists for the argument in phylogeny and taxonomy of this genus (family) (Pan, 1995). Embryological studies of peonies are briefly focused on the early embryogenesis as it is very peculiar in angiosperms for the free nuclear proembryo (Yakovlev and Yoffe, 1957; Murgai, 1959; Cave et al., 1962; Mattiessen, 1962; Carniel, 1967; Mu and Wang, 1985). Some works about the ovule and embryo sac of *Paeonia*, such as the megasporogenesis and gametophyte development of *P. californica* (Walter, 1962) and the vascular supply and ovule structure of *P. lactiflora* (Camp and Hubbard, 1962), have been done in herbaceous peonies, but there is lack of studies on woody species or tree peonies. China is the primary differentiation center of *Paeonia* and the tree peonies endemic to this country are especially significant to academic subjects related to this genus (Pan, 1995).

This paper dealt with the development of ovule and embryo sac, particularly the ovule ontogenesis and nucellar tissue differentiation, in *P. rockii*, a newly-identified tree peony (Hong. et al., 1992). This species is called as the blotched tree peony from which a larger cv. group has developed in Gansu province of northwest China and is thought as a very good germplasm resource for cv. improvement of tree peonies in future (Cheng and Li, 1994). The purpose of this work was to supply more embryological references for botanists to study *Paeonia* as well as for breeders to use effectively blotched tree peony in crossing.

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Materials and Methods

Ovules of *P. rockii* 'Mei Qui Sa Jin' (Rose Sprinkling Gold, with the purple double flower) and 'Hui Die' (Grey Butterfly, with the blended single flower), collected in the garden of Northwest Normal University in Lanzhou of China, were fixed and conserved in FAA solution (formalin: acetic acid: 50% ethanol, 1:1:18, v/v/v), hydrated in ethanol series and embedded in paraffin (melting point, 58-60 °C). Serial sections were cut at 8-15 μ and mainly stained with iron or Herlich's hematoxylin and some with Periodic acid-Schiff (PAS) reaction. Observation and photograph were carried out by Olympus Vanox microscope.

Results

The ovule, borne on the placenta of the ovary and comprised of the nucellus and the integuments subtending the nucellus, is the seat of megaspore formation and embryo sac or megagametophyte development. In blotched tree peony, the gynoecium differentiation and ovary formation occurred with the sprouting of plant in spring on the ground of carpel primordia formed in last autumn. When the carpels developed to close through a growing together (concrescence) of the ventral surfaces along the margins that were in contact with each other, the ovule began its ontogenesis.

1. The differentiation of ovule primordium

At the places of 275 μ far from the margins of original carpel along the two sides of the ventral suture of ovary, the ovule primordia began to differentiate acropetally at the distance of 330-340 μ from the base (Fig. 1, 1). Firstly the anticlinal divisions occurred in the cells of epidermis and sub-epidermis and then the cells of inside third layer divided periclinally much, which resulted in a protrusion or ovule primordium. Commonly in each ovary there were about 16-22 primordia located regularly in two lines along the ventral suture. Newly-formed primordia were a cylindrical structure with a rounded apex of about 150 μ in diameter and two periclinal cell layers in the surface were very evident for the frequently anticlinal divisions in these cells (Fig.1, 2).

2. The differentiation and formation of ovule

Having elongated to about 250 μ, the apex of ovule primordia curved to the ovary wall of one side and was followed by an evident nick beneath the apex where the inner integument protruded soon. With the appearance of inner integument, the central part of primordium became the primary nucellus tissue, in which the cells of epidermis and sub-epidermis underwent earlier a periclinal division separately (Fig. 1, 3). The inner integument mostly resulted from the anticlinal divisions of epidermal cells and a little lately the outer integument formed in the same way (Fig. 1, 4). As the integuments grew and the nucellus enlarged, the ovule continued curving while a series of events occurred in the nucellus. From the periclinal divisions of epidermis and sub-epidermis cells, an epistase formed at the tip of the nucellus and it consisted of the radial cell rows, 2-4 cells (most 2) wide and 4-6 cells (most 4) thick. Then the cells below the sub-epidermis divided in various planes to transfer into the archesporial cells forming a multicelled archesporium, a relatively temporal tissue, which formed the parietal and sporogenous cells by the periclinal divisions. At last in the flanks of nucellus between the archesporium and the sub-epidermis, a few parenchyma cells called the lateral cells were very distinct from the archesporial cells with denser protoplasm (Fig. 1, 4; 5).

With the differentiation within nucellus, the base of ovule extended into the funciculus, where the anticlinal walls were evidently extended in some epidermis cells close to the placenta. And these cells developed into the glandular cells further. The differentiation of glandular cells was accompanied with the development of a aril at the base of funciculus and was a morphological signal that the aril began developing and the ovule finished the tissue differentiation.

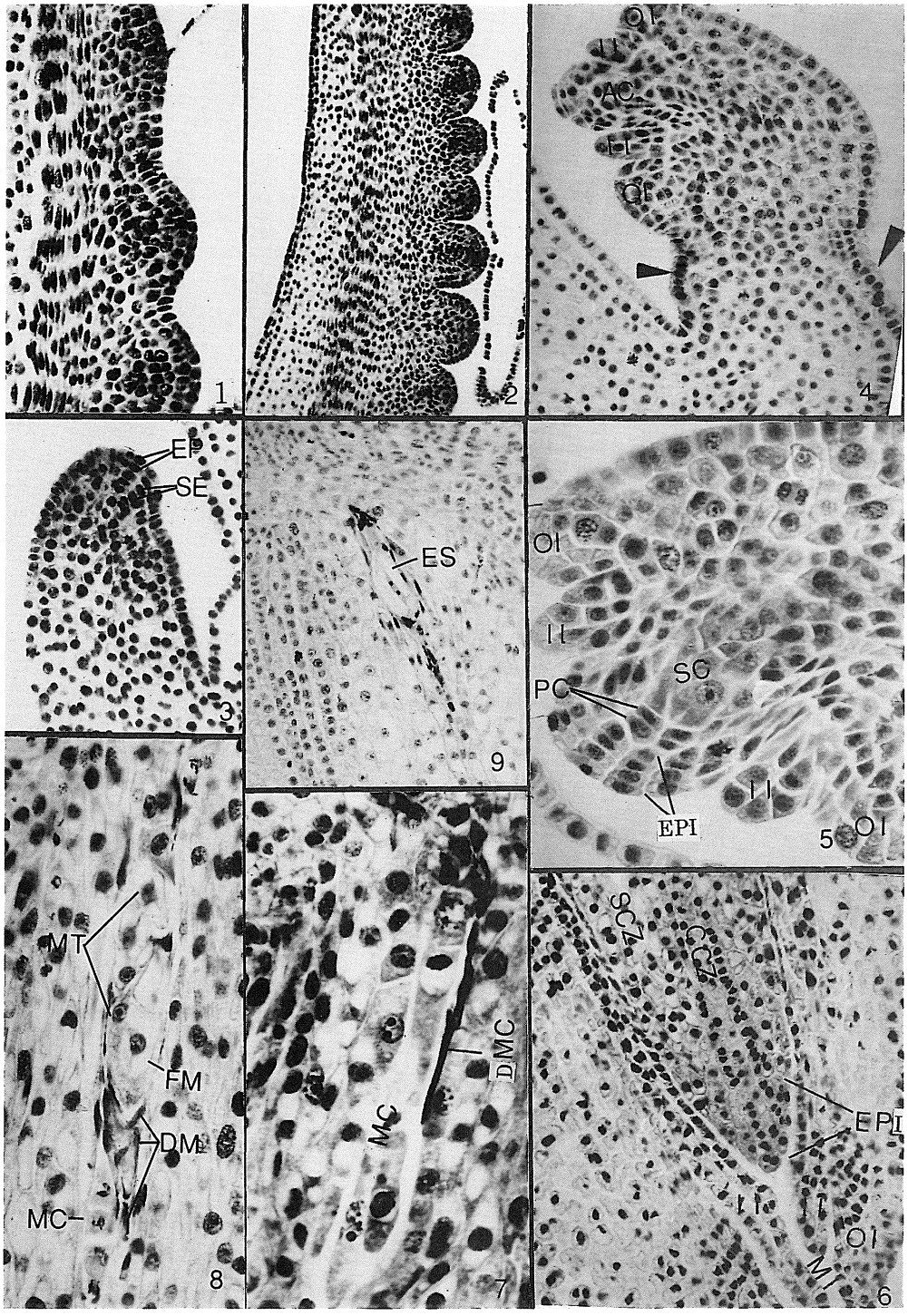

Fig. 1 Development of ovule and embryo sac in *P. rockii* (I).

1. Ovule primodia in differentiating acropetally; 2. Newly-formed ovule primodia; 3. The elongated primodium with the curving apex, showing the periclinal divisions of EP and SE cells; 4. II, OI, AC and funciculus (arrows) are developing; 5. EPI is formed and AC has divided into PC and SC; 6. The structure of II , OI, MI and nucellus; 7. MC in meiosis and DMC in the same ovule; 8. Showing the highly asynchronous meiosis, MC in meiosis, MT and FM can be seen; 9. A developing ES and the degenerated cells around it. All sections are stained with Herlich's hematoxylin solution. 1, 5,7 and 8 are magnified 300X, 2 is 75X and 3, 4, 6 and 9 are 150X. (AC: Archesporium; DM: Degenerated megaspore; DMC: Degenerated megasporocyte; EP: Epidermis; ES: Embryo sac; FM : Functional megaspore; LC: Lateral cell; MC: Megasporocyte; MT: Megaspore tetrad; PC: Parietal cell; SC: Sporogeneous cell; SE: Subepidermis; CCZ, EPI, II, MCZ, MI, OI and SCZ refer to Fig. 3.)

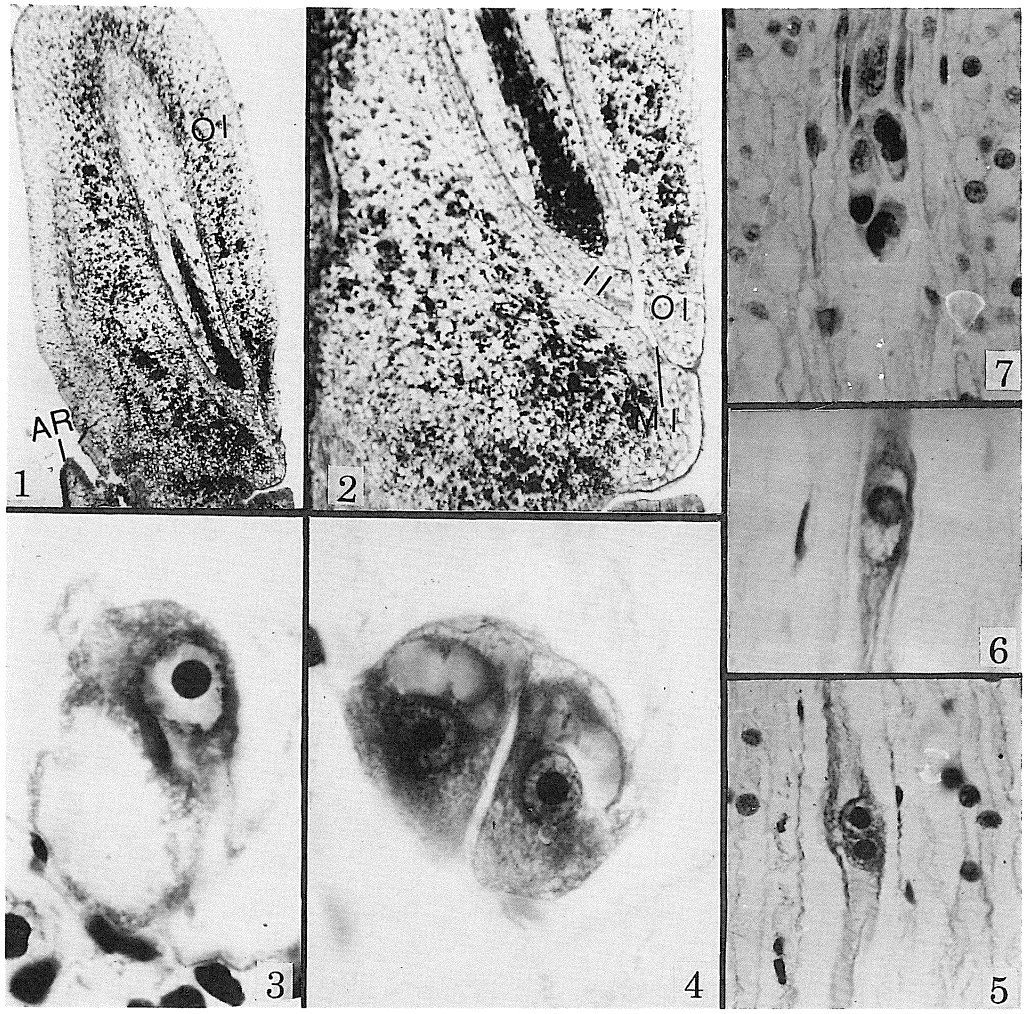

Fig. 2 Development of ovule and embryo sac in *P. rockii* (II).
1. Showing the basic structure of ovule and the distribution of starch gains; 2. The magnification of the micropyle end in 1; 3-7. The members of embryo sac before the fertilization, showing the egg in 3, synergids in 4, polar nuclei in 5 or secondary nucleus in 6 and antipodals in 7, respectively. The lower in 4-7 is the micropyle end, 1 and 2 are stained with Periodic acid-Schiff (PAS) reaction and the others with Herlich's hematoxylin solution. 1 is magnified 30X, 2 is 75X, 3 and 4 are 750X, and 5-6 are 300X. (AR, II, OI and MI refer to Fig. 3.)

3. Ovule growth and the formation of cyto-histological zones in nucellus

Blotched tree peony had typical anatropus, bitegmic and crassinucellate ovule. As the nucellus inverted completely, its apex turned toward the funiculus and was enveloped by both inner and outer integuments constituting the micropyle at the top together (Fig. 1, 6). The inner integument was always 2-3 cells thick, but the outer integument became thicker and thicker with the development of ovule until fertilization.

Just below the epistase in nucellus, the parietal cells derived from archesporial cells divided periclinally again and again to produce many secondary parietal cells arranging regularly in radial rows layer by layer. Meanwhile the inside sporogenous cells under parietal cells also underwent frequently periclinal divisions and formed 2-3 radial cell rows throughout nucellus. A little lately, the radial arrangement of these cells became obscure from the chalazal to micropyle ends gradually. The 1/3 cells of chalazal end increased evidently cytoplasm to become sporocytes forming a special cytohistological zone, the megasporocyte zone (MCZ). And the 2/3 cells of micropyle end were still parenchyma cells, which, with the radial rows of parietal cells,

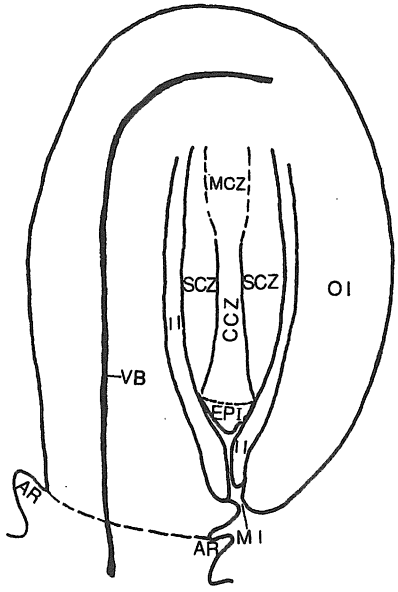

Fig. 3 Ovule structure of *P. rockii*.
(AR: Aril; CCZ: Central cell zone; EPI: Epistase; II: Inner integument; MCZ: Megasporocyte zone; MI: Micropyle; OI: Outer integument; SCZ: Surrounding cell zone; VB: Vascular bundle.)

constituted another cytohistological zone, the central cell zone (CCZ). Cells with different origins in the CCZ were distinct in morphology. Those from the parietal cells were smaller, lined more regularly in radial rows (Fig. 1, 6) and were filled with starch gains in the sections stained with PAS reaction (Fig. 2, 1; 2). Around the MCZ and CCZ, a surrounding cell zone (SCZ) composed of parenchyma cells developed from the derivative cells of the lateral cells in the flanks of nucellus (Fig. 1, 6; Fig. 3). Thus a complete ovule was established (Fig. 3) and its differentiation and formation were concluded as in Fig. 4. In such a ovule, starch gains were distributed regularly in different portions (Fig. 2, 1; 2). They filled in outer integument, epistase and CCZ, especially rich in latter twos, and, whereas, did not exist in the MCZ, SCZ and inner integument. However, it should be noted that at the chalazal end of CCZ the small part originated from sporogeneous cells in ontogeny had no distribution of starch gains.

4. Ovule development, megasporogenesis and embryo sac formation before anthesis

After being established, the ovule continued to change intensely with the megasporogenesis and the formation of embryo sac or megagametophyte. At anthesis, the outer integument is 320 μ thick, twice of the nucellus, but the inner integument was very unclear and only 2.5 μ in thickness; the cells in CCZ had entirely degenerated and disappeared so that the location was replaced by embryo sac; the cells in SCZ were vacuolarized highly while some cells close to embryo sac degenerated as well.

The boundary of MCZ was not precisely delineated because the adjacent parenchyma cells often joined megasporocytes with sporogenesis. The num-ber of sporocytes certainly varied from ovule to ovule but it was estimated to lie probably between 10 and 20 per ovule. Meiosis was evident firstly in the micropyle portion of MCZ and then proceeded toward the chalazal end, but, before long, such orderly progression disappeared and the various stages were seen scattered throughout the sporo-cytes. That was,

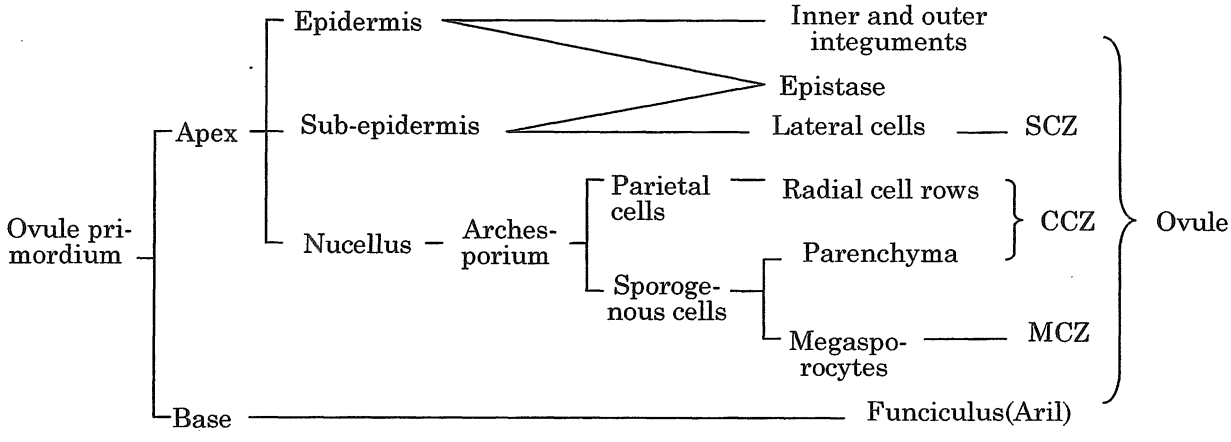

Fig. 4 Differentiation and formation of ovule in *P. rockii*.
(SCZ, CCZ and MCZ refer to Fig. 3.).

meiosis was highly asynchronous so that it was very often in an ovule that, while tetrad or functional megaspore or even gametophyte had formed in some cases, meiosis was undergoing or just in the onset in other cases. In the same ovule, some of sporocytes began meiosis, whereas others began to degenerate. Hence the new entrance into meiosis or the degeneration always accompanied sporocytes throughout sporogenesis, but no new meiosis started after the advanced gametophyte reached the stage of 4 free nuclei (Fig. 1, 7; 8). A linear tetrad was produced from meiosis and the chalazal megaspore was functional in most cases.

Embryo sac developed by *Polygonum* type. With the elongation of embryo sac toward the micropyle end, most nucellar cells in CCZ were withdrawn (Fig. 1, 9) so that the embryo sac was eventually elongated very much and directly reached the epistase. At last, the embryo sac was a slender structure with wider ends and thinner central portion, its length was decades of times of the width, and the widest at the micropyle end was about 60 μ but the thinnest at the central portion was not beyond 20 μ.

During sporogenesis and embryo sac development, most sporocytes entering meiosis degenerated successively at various stages and only 1-3 advanced ones got the priority in developing could finish the development entirely. In 100 ovules investigated after anthesis, the number containing 1, 2 and 3 embryo sacs was 49, 46 and 5 respectively, which showed that the ovule contained 1 or 2 embryo sac commonly and only 3 ones in a few cases.

5. The mature of ovule and embryo sac after anthesis

On the day at anthesis, the development of embryo sac varied very much by ovules and was probably in any stages from 4 nuclear to 8 nuclear or 7 celled. With the central nucleus and even cytoplasm, egg and synergids did not appear a polarity in the structure, even in the 7-celled embryo sac. Obviously such an embryo sac had no ability to accept pollens, even though the latter had matured and spread with the opening of flower, which, as a device, guaranteed the cross-pollination. While the embryo sacs belated in developing degenerated to disappear entirely within 1-2 days after anthesis, the remained ones tended to accord with each other in their development. As usual, so-called egg apparatus was differentiated at the micropyle end, antipodal cells at the chalazal end enlarged limitedly or proliferated into a multinuclear condition and polar nucleus fused often (Fig. 2, 3-7). The nucleus and cytoplasm of egg moved to the chalazal end and several larger vacuoles or a large one were located in the micropyle end (Fig. 2, 3). By contrast, the nucleus and cytoplasm of synergids were concentrated at the micropyle end with the formation of a filiform apparatus (Fig. 2, 4). On the third day after anthesis, one of synergids degenerated and all polar nucleus fused into the secondary nucleus (Fig. 2, 5; 6), so that the embryo sac was ready for fertilization. At this time, the glandular epidermis of aril had developed fully and excreted a large amount of exudation filling the ovary and soaking the ovules, while similar fluid was appeared on the surface of stigmas. These facts showed that embryo sac, ovule and pistil matured simultaneously, which could be identified simply by the active excretion of aril epidermis cells.

Discussion

In angiosperms, ovule primordium is produced from the periclinal divisions in the second or third layer cells of placenta and is divided into two types, bi-zonate and tri-zonate primordia. In the latter, the primordium is originated from the periclinal divisions of the third layer cell in placenta meristem whose epidermal and sub-epidermal cells only undergo the anticlinal divisions firstly. This is the basic type of angiosperms and widely occurs in some more primitive families in phylogenesis such as *Papaveraceae*, *Ranunculaceae* and *Magnoliaceae* and others (Bouman, 1984). The genesis of ovule primordium in blotched tree peony was typically belonged to the tri-zonate type and, however, the formation of archesporial and sporogenous cells and the differentiation of nucellus were very distinct from other

plants. In other angiosperms, the archesporial cells are located directly beneath the epidermis and divide periclinally into outer parietal cells and inner sporogeneous cells. The former divides repeatedly into a parietal tissue consisted of 1-10 or more layers of cells, which varies by species, and the latter directly functions as sporocyte (Bhojwani and Bhatnagar, 1979; Bouman, 1984). In tree peony, the archesporial cells occurred deeply inside under sub-epidermis and equally underwent the periclinal divisions to form parietal and sporogenous cells. However, the sporogeneous cells also divided repeated-periclinally like the parietal ones and their derivatives, except those functioning as sporocytes in the chalazal end, lined in radial rows in the center of nucellus and constituted a central cytohistological zone with the parietal cells together. That was, in tree peony the parietal cells as well as the sporogeneous cells joined the nucellus tissue, not as in other plants there is only the contribution of parietal cells. According to commonly-accepted view, the crassinucellate ovule is more primitive. When sporogeneous cells are specialized in their function and act directly as megasporocytes, the tenuinucellate ovule is formed. Therefore, we thought that the special pattern by which the crassinucellate ovule of tree peony developed was more primitive than the common ovule, because it would have no difference from the crassinucellate ovule of other plants if the archesporial cells were specialized and only could develop into sporocytes.

Multicelled archesporium, multiple megasporocytes and their asynchronous meiosis, anatropous, bitegmic and crassinucellate ovule and other related characters in blotch tree peony were the same as reported in herbaceous peonies (Johri, 1992; Walters, 1962) and, as the common features of the family *Paeoniaceae*, they were more primitive on the whole. We felt that to deal seriously with the characteristics appeared in the development of ovule and embryo sac should be very helpful to discuss the phylogenesis of *Paeoniaceae* by embryological data (Johri et al., 1992; Pan, 1995).

In the cross experiment of tree peony, we noticed that, as soon as the flower opens, the anther has matured completely and dehisces to spread pollens, but, at that time, hand cross-pollination was often unsuccessful. By this study, the reason for this phenomenon was that ovule and embryo sac were still not matured in physiology as well as in anatomical structure. In the ovule at anthesis, the embryo sac just differentiated all of elements and entered 8-nuclear or 7-celled stage or even still in developing by *Polygonum* type. In several days after anthesis, while the anthers had withered, the ovule and embryo sac matured entirely and were ready for accepting pollens, with the disappearance of belated embryo sac in the ovule, the appearance of polarity in egg and synergids, and the rapid development of glandular epidermis in aril. It is such cross-mature of gynoecium and androecium that supplies a device to guarantee the cross-pollination of tree peony in natural condition, which is beneficial to the natural evolution of this plant as well as the formation of such many cultivars in gardens.

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