Journal of Plant Biology, March 2008, 51(2) : 116-121

Pre-zygotic Embryological Characters of *Platycrater arguta*, a Rare and Endangered Species Endemic to East Asia

Cheng Qi Ao
School of Life and Environmental Sciences, Wenzhou University, Zhejiang 325027, China

***Platycrater arguta* Sieb. et Zucc. is a rare and endangered species endemic to East Asia. It produces two floral morphs viz. bisexual and male flowers. For bisexual flowers, simultaneous cytokinesis in the microsporocyte meiosis leads to a tetrahedral tetrad. The mature pollen grain is shed at 2-cell stage. The young anther wall is composed of epidermis, endothecium that develops fibrous thickenings at maturity, 1-2 middle layers and tapetum. The tapetum with uninucleate to binucleate cells, disintegrates in situ (glandular tapetum), yet in a small percentage of the anthers (about 37.6%), the tapetum does not disintegrate, causing complete male sterility. The ovules are anatropous, unitegmic, tenuinucellar and the formation of the embryo sac follows the monosporic, Polygonum type. Antipodal cells are lacking in the mature embryo sacs. Before fertilization, two polar nuclei fuse into a secondary nucleus. The formation of microsporangial wall, microsporogenesis and male gametogenesis in male flowers are analogous to those in the bisexual. Prezygotic embryological characters of *P. arguta* were reported for the first time, revealing that its endangerment is correlated with the abortion of pollen of a part, but not to the female development that is normal.**

Keywords: anther wall, endangerment mechanism, gametogenesis, *Platycrater arguta*, rare and endangered, sporogenesis

Human alteration of the global environment has caused major changes in the abundance and distribution of organisms (Liu et al., 2006). Most forest ecosystems in the world were fragmented by logging and other human disturbance, resulting in extinction of some species. While some of the other species survived habitat destruction, the number of populations as well as the number of individuals diminished severely, thus are threatened and in danger.

Platycrater Sieb. et Zucc. is a monotypic genus in Hydrangeaceae that includes *P. arguta*, a rare and endangered species endemic to *East* Asia (Cronquist, 1981; Fu, 1989). It is discontinuously distributed in East China (Zhejiang, Anhui, Jiangxi and Fujian) and Japan, in an altitude between 200-600 m (Fu, 1989). The plant is a perennial and deciduous shrub, 0.5 -1m in height (Fig. 1A). Its Chinese name "Spiderweb sepal" was derived from three prominent sepals in the male flower (Fig. 2A-F), which develop spiderweb-like venation after the flower withers. Due to its disjunctive distribution in China and Japan, it has important value in elucidating the properties and characters of flora of East Asia. Because of over logging and development of tourism, its habitat was seriously fragmented and it is now restricted to sunless steep cliffs near clean water (Fig. 1A). The populations are sparsely scattered in the above -mentioned provinces and very few individuals were encountered in a recent exhausting expedition. This species is at risk of extinction, and should be listed as endangered. Early in the year 1994, it was involved in the priority list of species of the greatest conservation concern in the Chinese Biodiversity Action Plan.

Information on prezygotic embryology of an endangered species is useful for understanding its endangerment mechanisms and planning management actions for conservation. Anther wall formation, sporogenesis and gametogenesis are key steps for determining a successful sexual reproduction. Nevertheless no embryological studies concerning these aspects have been conducted towards this species and the endangering mechanism of *P. arguta* is poorly understood. Therefore, the objective of the present research was to carefully examine those three developmental stages in *P. arguta* to clarify the cause of its difficult regeneration.

*Corresponding author; fax +86-577-88373034
e-mail aocq@wzu.edu.cn

MATERIALS AND METHODS

Floral buds at successive developmental stages were collected from a shrub that naturally grows in Dalongqiu scenic spot, Yandang mount, Zhejiang, China. Vouchers (Ao Chengqi 06010) were deposited in the Herbarium of Wenzhou University (WZU). These materials were fixed and stored in FAA (5 mL formalin: 6 mL acetic acid: 89 mL 50% ethanol), embedded in paraffin using conventional methods and sections with a thickness of 4 -11 μm were made. Stained with Heidenhain's haematoxylin, combined with safranin or fast green if necessary, the sections were observed and photographed with Lucky black and white film (SHD100) under an Olympus BH-2 microscope. Also, the permanent sections were deposited in WZU.

Pollen fertility was estimated according to the method of Majumdar et al. (2004), with propionocarmine replaced by acetocarmine. The naturally dehisced anthers were mounted on slides and at least 200 pollen grains for each anther were examined. The numbers of fully stained pollen grains as well as hyaline grains were counted in the optical field to determine the percentage of fertile grains.

RESULTS

This plant is andromonoecious and flowers aggregate in sparse corymbs (Fig. 1B). The bisexual flower possesses four aposepalous sepals and four apopetalous petals. Its andro-

Figure 1. *P. arguta* and its habitat. **A** *P. arguta* and its habitat. Scale bar = 400 mm. **B** a part of the plant. Scale bar = 20 mm.

ecium consists of numerous stamens. The ovary is bicarpellate, syncarpous and unilocular with two parietal placentae, while the style is dichotomal (Fig. 1B). The male flower has three synsepalous sepals (Fig. 2A-F), and its petals are incorporated as a whole (Fig. 2G). The male flower, involving a number of stamens (Fig. 2G), was once misidentified as a sterile flower. The following observations refer to the bisexual flowers. Formation of anther wall, microsporogenesis and male gametogenesis in male flowers are analogous to those in the bisexual, and are not reported here.

Formation of Anther Wall

The anther of *P. arguta* is tetrasporangiate (Fig. 3F). At an early stage of development, rows of archesporial cells differentiate beneath the epidermis of the anthers and divide periclinally, forming outer primary parietal cells and inner primary sporogenous cells (Xue and Li, 2005).

The primary parietal cells divide periclinally and anticlinally, forming two layers of secondary parietal cells (Fig. 3A). The outer secondary parietal cells form a subepidermal endothecium and 1-2 middle layers (Fig. 3B). The middle layers have a common origin with the endothecium, so the microsporangial wall formation follows the Dicotyledonous Type (Davis, 1966; Xue and Li, 2005). The inner secondary parietal cells give rise to part of the tapetum. The anther wall consists of 4-5 layers; from the exterior, the epidermis, endothecium, 1-2 middle layers and tapetum (Fig. 3B). The epidermis is a single-layered external covering of the sporangial wall, only relicts of which are detected after the anther wall has ripened (Fig. 3E). The tapetal cells are uninucleated or binucleated at the stage of pollen mother cells (PMC), and they disintegrate in situ at the stage of one-nucleate microspores (Fig. 3C). Thus this antheral tapetum belongs to glandular type. The middle layers have degenerated at the stage of one-nucleate microspores (Fig. 3C). The mature anther wall is only of endothecium and it develops fibrous thickenings (Fig. 3E). PMCs and microspores develop normally, as a result of disintegration of tapetum (Fig. 3C). Pollen grains are all aborted, provided the tapetum does not disintegrate (Fig. 3D). The aborted grains, anuclear and irregular in shape, are distinguishable from normally-developed grains (inserted figures in Fig. 3C, D). Out of 250 sporangia examined, 94 (37.6%) were found to possess non-disintegrative tapeta.

Microsporogenesis and Male Gametophyte Development

The primary sporogenous cells derived from archesporial cell differentiation produce through mitosis secondary

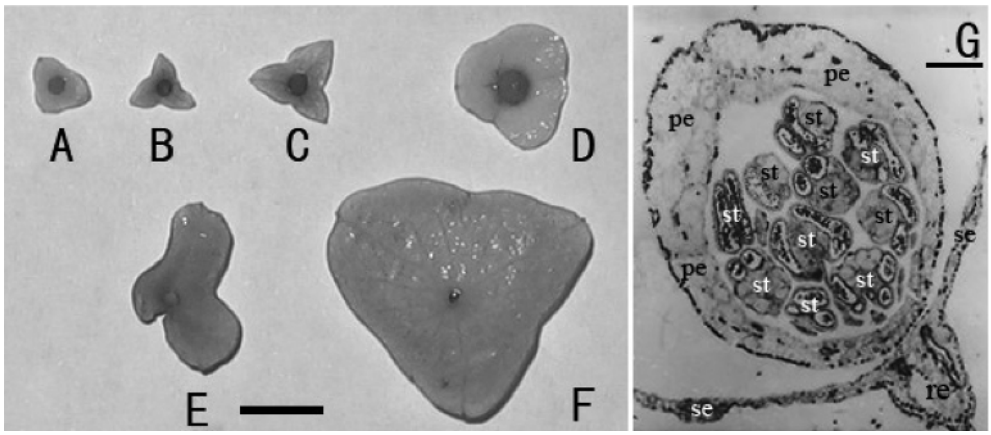

Figure 2. A -F Male flowers at different developmental stages. Scale bar = 5 mm. **A -D** Male flowers with androecium. **E-F** The androecium has left the male flower. **G** Longitudinal section of a male flower. Scale bar = 400 μm. re: receptacle, se: sepal, pe: petal; st: stamen.

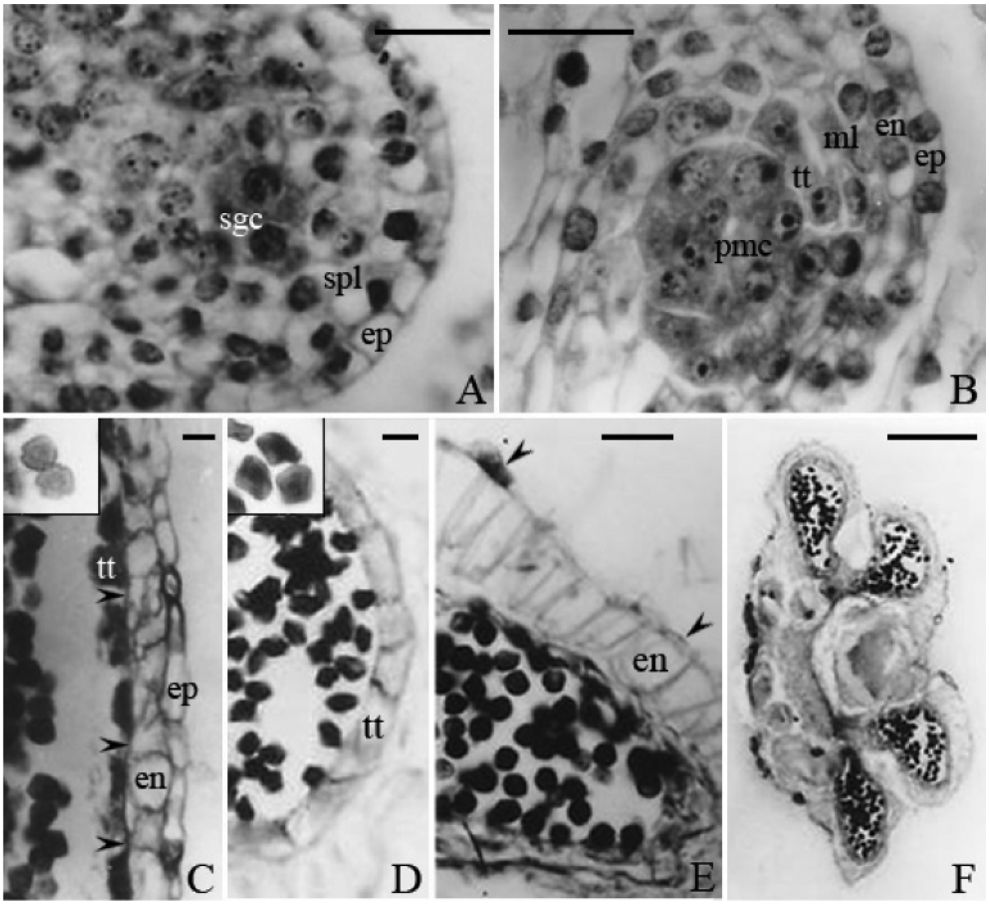

Figure 3. Formation of anther wall. ep: epidermis, spl: secondary parietal cells, sgc: sporogenous cells, en: endothecium, ml: middle layer, tt: tapetum, pmc: pollen mother cell. **A** Anther wall consisting of epidermis (ep), secondary parietal cells (spl) (two layers) and secondary sporogenous cells (sgc). Scale bar = 20 μm. **B** The outer secondary parietal has divided into endothecium (en) and middle layers (ml), showing Dicotyledonous Type of wall formation. Anther wall is composed of epidermis, endothecium, one or two middle layers and tapetum (tt). Secondary sporogenous cells have developed into pollen mother cells (pmc). Scale bar = 25 μm. **C** Tapetum is disintegrating in situ, giving rise to normal uninucleated pollen grains (inserted figure). Note that the middle layer has degenerated (arrows). Scale bar = 10 μm. **D** Tapetum does not disintegrate, causing abortion of pollen grain. Inserted are anuclear aborted pollen grains, irregular in shape. Scale bar = 15 μm. **E** Mature anther wall of fibrous thickenings. Arrows indicate the relict of epidermis. Scale bar = 40 μm. **F** A tetrasporangiate anther. Scale bar = 140 μm.

sporogenous cells that develop into PMCs (Fig. 4A). Meiosis in the PMCs leads to tetrahedral tetrads (Fig. 4B). Sometimes callose is well discerned around the tetrad and between each monad. No cell wall was detected between the two newly -formed nuclei at telophase I (Fig. 4A). Clearly the cytokinesis is of the simultaneous type. PMCs in the same sporangium synchronize in development, but those in two different sporangia do not, with one lagging 1-3 stages behind the other. The microspore just released from tetrads has no vacuole, yet it has a dense cytoplasm and is somewhat irregular in shape (Fig. 4C). Then it is vacuolated (Fig. 4D), but soon afterwards the vacuole disappears. The microspore then divides by mitosis into two unequal cells, viz. a large vegetative and small reproductive one thus to bring a 2-cell pollen grain (Fig. 4E). The 2-cell pollen grain has archshaped cell wall between generative and vegetative cells (Fig. 4F). Then the generative cell enters the cytoplasm of the vegetative cell gradually (Fig. 4G, H). The reproductive cell does not undergo mitotic division and the pollen maintains two cells before anther dehiscence. Thus the mature pollen grains are two -celled (Fig. 4I).

Megasporogenesis and Female Gametophyte Development

The ovule primordium begins initiating from early March of each year (Fig. 5A). Later in April, it bends gradually outward due to more cell division at the outer side of the primordia than at the inner side (Fig. 5B). Shortly afterwards it differentiates into integument and nucellus (Fig. 5B). When the ovule turns anatropous, the megaspore mother cell (MMC) is apparent beneath the nucellar epidermis of one layer (Fig. 5C). Hereby the ovule is tenuinucellar. The MMC undergoes two successive meiotic divisions (Fig. 5D), giving birth to a linear tetrad (Fig. 5E). The chalazal megaspore is functional (Fig. 5E), thus developing into a mononucleate embryo sac (Fig. 5F), while the others degenerate (Fig. 5E). Clearly this developmental manner conforms to the Polygonum Type. The functional megaspore develops into a 2-nucleate embryo sac through the first subsequent mitotic division (Fig. 5G, H, I), a 4-nucleate embryo sac via the second (Fig. 5J, K). At the eight -nucleate stage, three nuclei at the micropylar pole constituted the egg apparatus, compris-

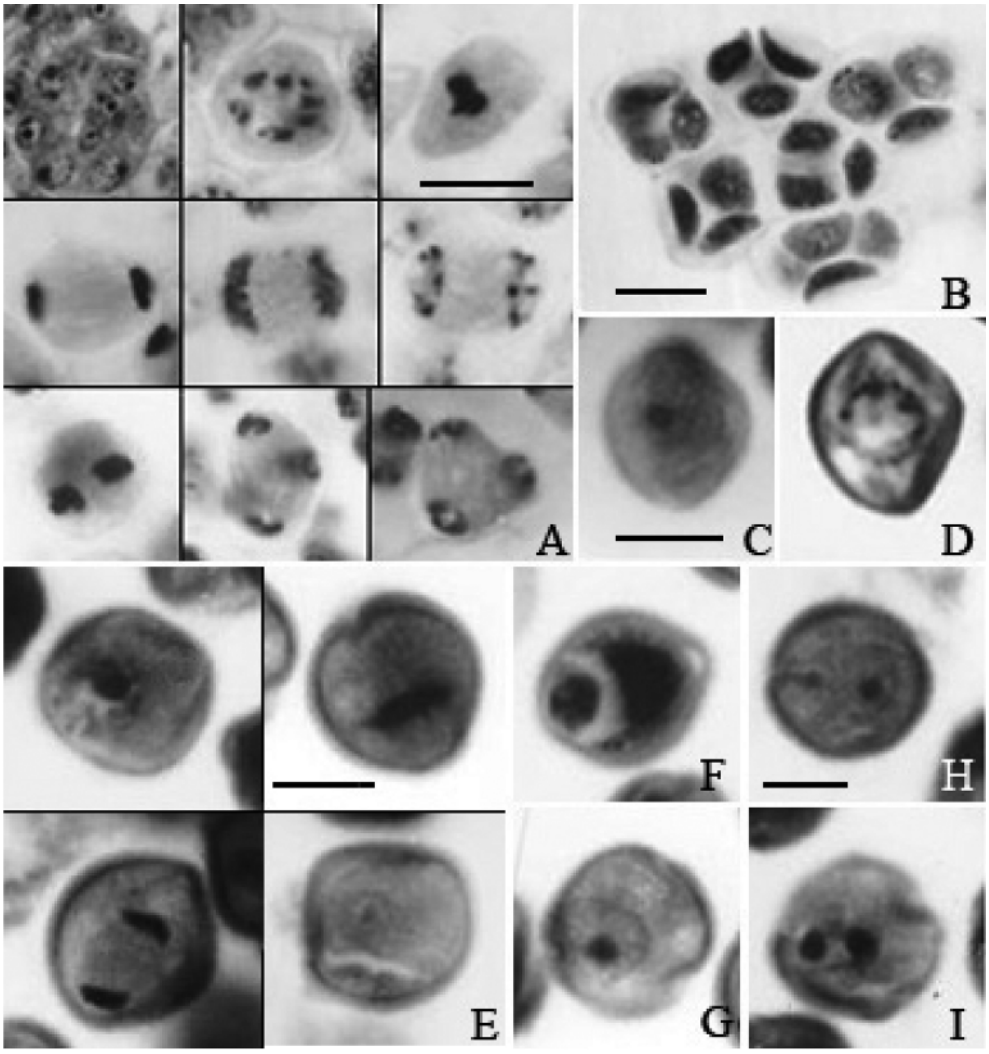

Figure 4. Microsporogenesis and male gametophyte development. **A** PMC and its meiosis. Scale bar = 10 μm. **B** Tetrahedral tetrads. Scale bar = 10 μm. **C** Uninucleate microspore just released from tetrad, with shrunken wall. Scale bar = 5 μm and it also applies to **D**. **D** Microspore was vacuolated. **E** The uninucleate microspore undergoes mitosis. Scale bar = 5 μm. **F** 2-celled pollen grain with archshaped cell wall between generative and vegetative cells. **G**, **H** The generative cell enters the cytoplasm of the vegetative cell gradually. **I** Mature pollen grain. Scale bar in **H** = 5 μm and also applies to **F**, **G** and **I**.

ing an egg cell (Fig. 5L) and two synergids (Fig. 5M). The polar nuclei usually fuse to form a secondary nucleus before fertilization (Fig. 5M, inserted figure). No antipodal cells were detected after a great many mature embryo sacs were examined (Fig. 5L, M).

Estimation of Pollen Fertility

In fully-developed anthers, the mean percentage of pollen stainability was 82.7%, indicating high pollen fertility, while in those whose tapetum does not disintegrate, no pollen grain was stainable, suggesting complete male sterility.

DISCUSSION

The theory of embryology can explain well endangerment mechanism of threatened species. As examples, Yin and Fan (1997) believed that pollen sterility was an important factor limiting production of *Liriodendron chinense*, most of which occurred before the tetrad stage, yet a minority happened after microspore formation. Pan et al. (2003) discovered that the germination rate of pollen under artificial conditions of *Manglietia aromatica* was as low as 0.01%. They claimed that this was obviously too poor to pollinate effectively. Xiao and Yuan (2006) thought that the poor regeneration of *Sinomanglietia glauca* was possibly caused by the stony seed coat either blocking water supply or producing unknown signals repressing seed germination under natural conditions.

Concerning *P. arguta*, in quite a few anthers, whether of bisexual flowers or of male flowers, the tapetum does not disintegrate, causing complete sterility of pollen grains. The tapetum is a highly specialized secretory cell layer responsible both for pollen cell wall deposition and for the production of locular fluid that supplies the nutrients required for pollen maturation (Clement et al., 1998). A recent study

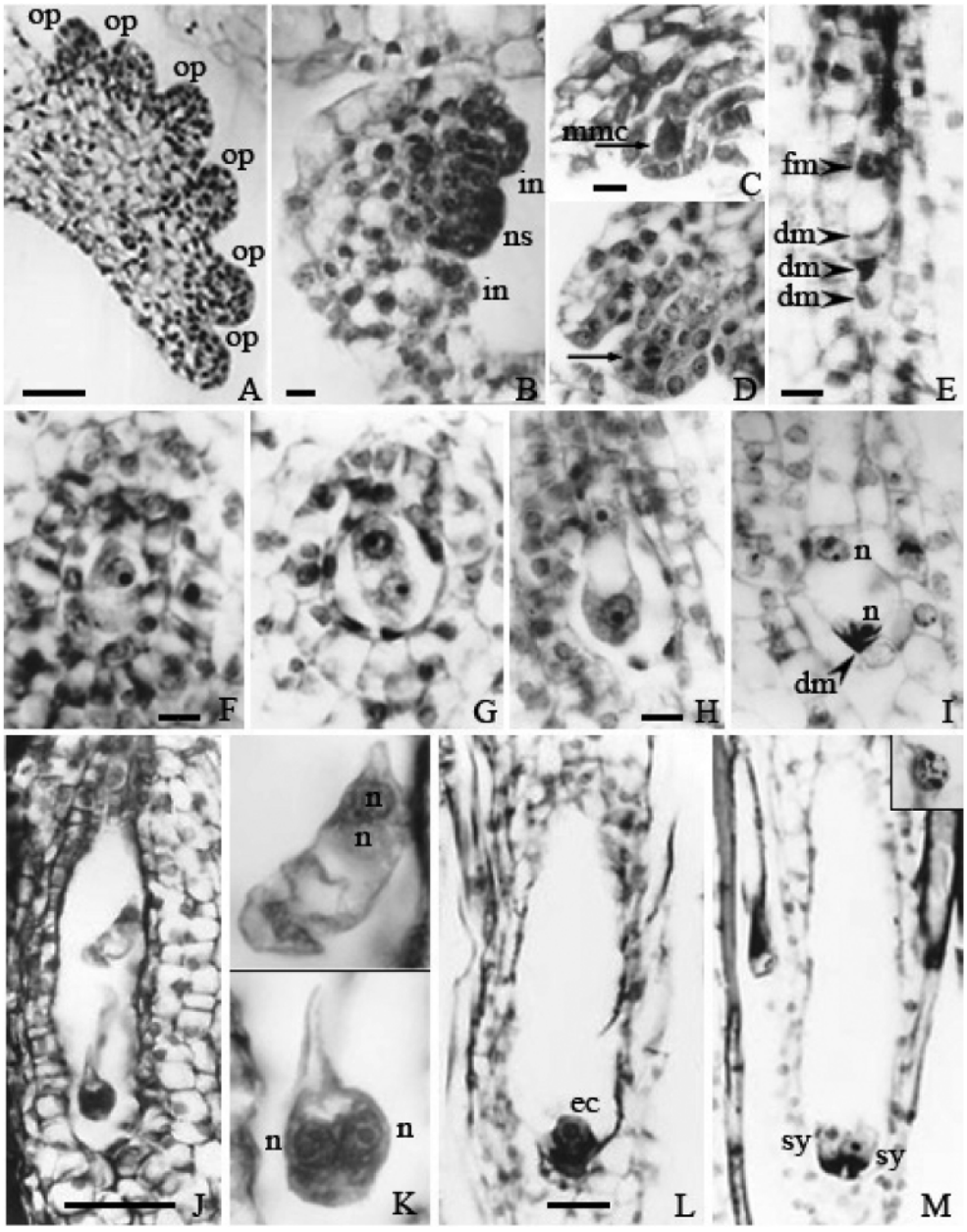

Figure 5. Megasporogenesis and female gametophyte development. The micropylar end is at the bottom of figures. op: ovule primordium, in: integument, ns: nucellus, mmc: megaspore mother cell, dm: degenerated megaspore; fm: functional megaspore; n: nucleus; ec: egg cell; sy: synergid. **A** Just-initiated ovule primordia (op). Scale bar = 40 μm. **B** Ovule initiating integument (in). Scale bar = 10 μm. **C** Megaspore mother cell (mmc). Scale bar = 10 μm, and also applies to **D**. **D** Anaphase I of meiosis in the MMC. **E** Megaspores at linear tetrad stage. Scale bar = 10 μm. **F** Mononucleate embryo sac. Scale bar = 10 μm. **G, H** Embryo sac at two-nucleate stage. Scale bar = 10 μm, and also applies to **I**. **I** Two-nucleate embryo sac and degenerated megaspores at the micropylar end, suggest that the development of the embryo sac follows the Polygonum type. **J** Four-nucleate embryo sac. Scale bar = 50 μm. **K** Enlargement of **J** shows two nuclei at the chalazal and micropylar end respectively. **L, M** Successive sections show an egg apparatus: **L** Egg cell. **M** Two synergids. Inserted is a secondary nucleus in the same embryo sac. Note antipodal cells lacked. Scale bar = 30 μm.

revealed that changes in the structure of endoplasmic reticulum (ER) of the rice tapetum are associated with tapetal abnormal functioning and, consequently, male sterility. For example, the concentric ER rings found at 16°C stress failed to support the secretory functioning of the tapetal cells, leading to the abortion of microspores (Gothandam et al, 2007). Whether this is the case in *P. arguta* remains unclear. However, it can be concluded that the abortion of pollen of a part is correlated to the endangerment of *P. arguta*, though the female development is normal.

For species on the brink of extinction, there is an urgent need for conservation (Maschinski and Duquesnel, 2006). Reintroduction was once encouraged as an extinction prevention strategy for plant species (Maunder, 1992; Falk et al., 1996), but examples of effective reintroductions that result in self -sustaining populations are limited (e.g., Griffith

et al., 1989). Taken into consideration the special habitat and ecological requirement of *P. arguta*, in situ conservation of natural populations and establishment of nature reserves are important strategies for conserving this species. A thorough survey on the distribution of *P. arguta* in *East* China is imperative. Based on allozyme, RAPD and ISSR techniques, those populations with higher genetic diversity should be selected to protect prior to others. Some insect species should be protected in situ or introduced and raised to raise pollination efficiency. Besides, manual harvest and sowing of seeds are also quite necessary for regeneration of individuals and recovery of populations (Qiu and Fu, 2001).

As a continuation of the present work, postzygotic reproductive studies must go forward so that this information becomes available as part of the equation when deciding how best to increase the survival chances of *P. arguta* (Mcmullen, 2007).

ACKNOWLEDGMENTS

I thank Prof. Ding Bingyang and Dr. Jin Xiaofeng for their valuable information on the distribution of *P. arguta*. This research was supported by the Young Teacher Plan in Higher School in Zhejiang Province.

Received November 5, 2007; accepted January 7, 2008.

LITERATURE CITED

Clement C, Laporte P, Audran JC (1998) The loculus content and tapetum during pollen development in *Lilium*. Sex Plant Reprod 11: 94-106

Cronquist A (1981) An Integrated System of Classification of Flowering Plants. Columbia University Press, New York

Davis GL (1966) Systematic Embryology of the Angiosperms. John Wiley & Sons, Inc., New York, London, and Sydney

Falk DA, Millar CI, Olwell M (1996) Restoring Diversity: Strategies for Reintroduction of Endangered Plants. Island Press, Washington, DC

Fu LG (1989) Rare and Endangered Plants in China. Shanghai Education Publishing House, pp 305-307

Gothandam KM, Kim ES, Chung YY (2007) Ultrastructural study of rice tapetum under low-temperature stress. J Plant Biol 50: 396-402

Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool: Status and strategy. Science 245: 477-480

Liu P, Yang YS, Xu GD, Hao CY (2006) Physiological response of rare and endangered seven-son-flower (*Heptacodium miconioides*) to light stress under habitat fragmentation. Environ Exp Bot 57: 32-40

Majumdar S, Banerjee S, De KK (2004) Meiotic behaviour of chromosomes in PMCs and karyotype of *Trifolium repens* L from Darjeeling Himalaya. Acta Biol Cracov Series Bot 46: 217-220

Maschinski J, Duquesnel J (2006) Successful reintroductions of the endangered long-lived Sargent's cherry palm, *Pseudophoenix sargentii*, in the Florida Keys. Biol Conserv 134: 122-129

Maunder M (1992) Plant reintroduction: An overview. Biodivers Conserv 1: 51-61

McMullen CK (2007) Pollination biology of the Galápagos endemic, *Tournefortia rufo-sericea* (Boraginaceae). Bot J Linn Soc 153: 21-31

Pan YZ, Liang HX, Gong X (2003) Studies on the reproductive biology and endangerment mechanism of the endangered plant *Manglietia aromatica*. Acta Bot Sin 45: 311-316

Qiu YX, Fu CX (2001) Studies on the endangerment mechanism of and conservation strategies for *Changium smyrnioides*. Biodivers Sci 9: 151-156

Xiao DX, Yuan Z (2006) Embryogenesis and seed development in *Sinomanglietia glauca* (Magnoliaceae). J Plant Res 119: 163-166

Xue CY, Li DZ (2005) Embryology of *Megacodon stylophorus* and *Veratrilla baillonii* (Gentianaceae): Descriptions and systematic implications. Bot J Linn Soc 147: 317-331

Yin ZF, Fan RW (1997) Ultrastructure observation on the abortion of *Liriodendron chinense* pollen. J Plant Resour Envir 6: 1-7