Interspecific Hybridization Processes between *Michelia yunnanensis* and *M. crassipes* and Embryogenesis of the Heterozygote

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Abstract. The investigation of hybridization processes and embryogenesis of heterozygote is an effective approach for early hybrids' identification, which could provide reliable information for successful crossbreeding. In this study, we reported the whole hybridization processes of the direct cross and reciprocal cross between *Michelia yunnanensis* Franch. ex Finet et Gagnep. and *Michelia crassipes* Law using fluorescence microscopy after aniline blue staining, with the pollen germination on stigmas, pollen tube growth in styles, and subsequent extension into the embryo sac as well as the double fertilization processes are documented in detail. The *M. yunnanensis* × *M. crassipes* combination displayed considerable cross-compatibility, and the heterozygote embryogenesis was further observed with an approach of modified cryosectioning technique. Besides, the whole formation processes of hybrid seeds from artificial pollination to maturation were successfully observed. However, in the reciprocal cross, we found incompatibility between pollen grains of *M. yunnanensis* and stigmas of *M. crassipes* for the reason of hysteretic identification, as well as the abnormal callose deposition which belongs to the prefertilization barriers. This is the first study in which the complete and clear hybridization processes in *Michelia* were reported. We inferred that unilateral incompatibility of *M. crassipes* detected in this study may also exist in some other *Michelia* species. In artificial hybridization practices, we suggest some special treatments for overcoming prefertilization barrier should be taken when treating *M. crassipes* as the maternal parent.

Michelia L. is a large genus from the Magnoliaceae family and consists of about 80 tree- or shrub-habit species which can be characterized by axillary flowers, lateral dehiscent anthers, and obvious gynoeceum handle (Law, 2004). The flowers of *Michelia* are usually white or yellow, excepting *M. crassipes* Law, *M. moutan* Dunn var. *rubicunda* Yiet. J.C. Fan (Fan et al., 2009) and *M. rubriflora* Law et R.Z. Zhou (Wang et al., 2005), whose flowers are purple or red. Crossbreeding can create colorful hybrid cultivars. Thus, *M. crassipes* is widely

cultivated and highly desirable in breeding new cultivars for its unique purple blooms. In recent decades, a total of nine new *Michelia* interspecific hybrid varieties were derived from *M. crassipes* (Gong et al., 2003b; Han et al., 2014; Li et al., 2013; Shao et al., 2015a, 2015b; Shao et al., 2016). Intriguingly, in the above studies, *M. crassipes* was often treated as paternal parent, with the two cultivars Chilongzhua and Yanzhizui as exceptions, which employed *M. calcicola* as paternal and *M. crassipes* as maternal parent (Han et al., 2014). In addition, previous interspecific hybridization studies within *Michelia* also reported that incompatibility existed between the maternal *M. crassipes* and the other species in the genus (Wang et al., 2003), suggesting some interspecific hybridization with respect to *M. crassipes* may be unilateral (Lewis, 1958), videlicet, the cross using *M. crassipes* as a parent is only successful in one direction, whereas the reciprocal cross always fails.

We have carried out numerous artificial hybridization combinations and obtained a series of intra- and intergeneric hybrids from Magnoliaceae plants since 1996. One of the cross combinations was *Michelia yunnanensis* × *M. crassipes*, its three new horticulture cultivars Yujin, Danxin, and Qinfang have been evaluated and registered at Kunming by the forestry department of Yunnan province in 2002. The horticultural characters, cultivation and propagation techniques, and cytological identification of the new cultivars were recorded during crossbreeding following previous studies (Fu and Sun, 2006; Gong et al., 2001, 2003a; Ji and Sun, 2011; Pan et al., 2008). However, the karyotypes of *M. yunnanensis* × *M. crassipes* offspring were markedly different from the expected ones according to the parental karyotypes, which might result from the structural variation of the parental chromosome during the hybridization (Pan et al., 2008). Moreover, there was few reported study regarding the hybridization processes and heterozygote embryogenesis in *Michelia*, especially for the possible unilateral incompatibility of *M. crassipes*.

In this study, we further investigated the interspecific hybridization processes between *M. yunnanensis* and *M. crassipes* and their heterozygote embryogenesis, and we hereby addressed three questions: 1) What are the hybridization processes of the direct cross and reciprocal cross between *M. yunnanensis* and *M. crassipes*? 2) Is there unilateral incompatibility for *M. crassipes* in this combination, which could result in this phenomenon? 3) What theoretical evidence and practical guidance will the study provide for future interspecific hybridizations on *M. crassipes*?

Materials and Methods

Investigations were carried out on the bidirectional cross between *M. yunnanensis* and *M. crassipes*, cultivated in Kunming Botany Garden, Yunnan, China. The pollinated gynoeceia within 4 d after pollination (DAP) were used for observing pollen germination on the stigmas, pollen tube growth in the styles and subsequent extension into the embryo sac, as well as the double fertilization by fluorescence microscopy. The heterozygote embryogenesis of gynoeceia after 5 DAP was studied using the approach of modified cryosectioning technique.

Aniline blue fluorescence method. Artificial pollination was carried out according to the conventional artificial hybridization method (Jiang et al., 1999). Pollinated gynoeceia were collected hourly within 6 h after pollination (HAP), every other hour from the seventh to 12th HAP, and then every day from 1st to 7th DAP. The samples were fixed in FAA (70% ethyl alcohol/glacial acetic acid/formaldehyde = 90/5/5%) and stored in 4 °C. Pistils were divided into different portions according to the purpose of subsequent observations, e.g., stigma, style and ovary, or stigma and style together, and

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placenta with ovules. The fixed materials were macerated in 5% NaOH at 60 °C for 4 h after being rinsed with distilled water five times. After maceration, the materials were rinsed again and stained with 0.01% aniline blue for 24 h. Slides were mounted with glycerol and kept in darkness. Pollen grain germination and pollen tube growth were observed with a Leica DM5500 B fluorescence microscope and software LAS.V4.

Modified cryosectioning technique. Pollinated gynoecia were collected every day at the first week and every other day at the second week. Then young fruitlets were collected every week at the following weeks. The samples were fixed in FAA (70% ethyl alcohol/glacial acetic acid/formaldehyde = 90/5/5%) and then stored in 4 °C. The modified cryosectioning technique, involving alcohol decolorization, embedding with Leica

biosystems tissue freezing medium and application of Poly-1-lysine slides, was used in the observation of heterozygote embryogenesis process.

In detail, the first and key step was the preparation of Poly-1-lysine slides, which could prevent the tissue from exfoliating and being damaged in the process of sticking slices. Fruitlets were dehydrated via an ethanol series, 70% (4 h)—80% (2 h)—95%

Fig. 1. The germination of pollen grains of *Michelia crassipes* on the stigma of *M. yunnanensis* from 1 h to 4 d after pollination by the fluorescence microscope (yellow-green showed the pollen tube). (A) Pollen grains of *M. crassipes* adhered to the stigma of *M. yunnanensis*; (B) pollens of *M. crassipes* started germinating on the stigma of *M. yunnanensis*; (C–E) pollen tubes began to elongate into the style of *M. yunnanensis*; (F) pollen tubes merged into a bundle; (G and H) pollen tubes extended into the middle-low part of style; (I and J) pollen tubes bundle entered in the ovary; (K) pollen tubes bundle separated and reached each ovule; (L) double fertilization, yellow-green fluorescence showed a whole interspecies hybridization pathway between *M. yunnanensis* and *M. crassipes* from pollen grains germination to double fertilization. Scale bar = 500 μ m.

(2 h)—100% (1 h)—100% (30 min), and then embedded in Leica biosystems tissue freezing medium for microtoming. Serial sections were cut with a Leica freezing microtome CM 3050 S at a thickness of 8–12 μm and then were stained with 10% hematoxylin. All the microtome sections were observed with

Leica microscope DM5500 B and software LAS.V4.

Results

*Hybridization processes and heterozygote embryogenesis of *M. yunnanensis* $\times$ *M. crassipes*.*

Fig. 2. Comparison of fertilized ovule by the fluorescence microscope (yellow-green showed the pollen tubes). OV: ovule; (A) unfertilized ovules in the ovary of *Michelia yunnanensis*; (B) fertilized ovules in the ovary of *M. yunnanensis*; (C) fertilized ovules (chalazogamy) on the 10 $\times$ fluorescence microscope; (D) fertilized ovule (chalazogamy) on the 20 $\times$ fluorescence microscope. (A and B) Scale bar = 500 μm ; (C and D) scale bar = 100 μm .

The pollen grains of *M. crassipes* adhered to the stigma of *M. yunnanensis* at the first HAP (Fig. 1A), and started to germinate on the stigma 4 HAP (Fig. 1B), 2 h later than in vitro conditions with hanging drop culture method. Pollen tubes began to elongate into the style of *M. yunnanensis* from 5 to 8 HAP (Fig. 1C–E), then continued to grow and merged into a bundle 10 HAP (Fig. 1F). The pollen tubes extended into the middle and lower part of style during 12–24 HAP (Fig. 1G and H) and then entered into the ovary 48 HAP (Fig. 1I and J). The bundle of pollen tubes separated and reached each ovule 3 DAP (Fig. 1K). Remarkably, the pollen tubes penetrated the ovule through the chalazal rather than the micropyle for fertilization, which is the chalazogamy (Fig. 2A–D). Double fertilization was finished at the fourth day after pollination (Fig. 1L), with one of the two gametes fusing with the egg cell to form the zygote, and the other one

Fig. 3. Heterozygote embryogenesis of *Michelia yunnanensis* $\times$ *M. crassipes*. (A) Anatropous ovule; (B–D) black arrow showing the embryogeny morphology; (B) 4-cell proembryo; (C) multicellular embryo; (D) globular embryo; (E) cordate embryo; (F) torpedo embryo; (G–I) embryogeny of *M. yunnanensis* $\times$ *M. crassipes* from 25 to 45 d after pollination. (A – E) Scale bars = 50 μm ; (F) scale bars = 100 μm ; (G and H) scale bars = 200 μm ; (I) scale bar = 500 μm .

fusing with the central cell nucleus to form the primary endosperm nucleus.

The ovules of *M. yunnanensis* are crassinucellate, bitegmic, and anatropous (Fig. 3A). Without dormancy, the primary endosperm nucleus divided quickly to form many free nuclei before cell walls were laid down. Thus, endosperm development is the nuclear type. The fertilized embryo sac developed into a polygonum type. The zygote developed into a straight line-like 4-cell proembryo (Fig. 3B) 5 DAP, and then a multicellular proembryo (Fig. 3C) 8 DAP. The development of embryo could be divided into four stages: globular (Fig. 3D), cordate (Fig. 3E), torpedo (Fig. 3F), and mature embryo (Fig. 3G–I). It took about 4 months for hybrid seeds from artificial pollination to maturation (Table 1).

Hybridization processes of *M. crassipes* × *M. yunnanensis*. In the reciprocal cross, the stigma of *M. crassipes* could hardly identify the pollens from *M. yunnanensis* at the first 6 HAP (Fig. 4A–C). Only a small number of pollen grains germinated on the stigma after 8 HAP (Fig. 4D), 10 HAP (Fig. 4E), and 12 HAP (Fig. 4F). Although massive pollen grains were observed to have germinated at 24 HAP (Fig. 4G), and pollen tubes could merge into the bundle at 48 HAP (Fig. 4H) and extend into the style at 3 DAP (Fig. 4I and J), the pollen tubes discontinued the later extension and failed in reaching ovules for double fertilization because of abnormal callose deposition (Fig. 4K and L).

Discussion

Originality of the modified cryosectioning technique. Multiple protocols for cryosectioning for animal samples have been set up since the last decades, whereas few protocols were used for plants tissues (Risueno et al., 1998). The cryosectioning in plants is usually challenging because of the specific characteristics of plant cells and tissues (e.g., large vacuoles, cell wall, permeability problems, and differences in hardness) (Chen and Zhao, 2005; Michel et al., 1991; Zhang et al., 2008, 2012). Most of the studies on embryogenesis of Magnoliaceae or other plant species used the conventional paraffin sectioning (Fan et al., 1992; Wang et al., 2010; Xiao and Yuan, 2006). Although the further improvement in cryosectioning procedures could be available, the modified cryosectioning technique occupied in this study including alcohol decolorization, soaking in embedding medium, and application of polylysine could be treated as an alternative or more extensive applications to the traditional paraffin sectioning method for the embryological studies of *Michelia* species.

The interspecific cross-compatibility of *M. crassipes*. The genetic relationship of species could be reflected by the level of interspecific or intergeneric cross-compatibility (De Nettancourt, 2013). As an accessible method, the investigating of seed set, seed vitality, and growth situation of the hybrid seedlings was used to study the cross-compatibility of

Magnoliaceae (Gong et al., 2001; Wang et al., 2003). The results of 21 cross combinations within *Michelia* indicate that the cross-compatibility commonly exists in this genus (Gong et al., 2001). But Wang et al.,

(2003) found that hybridization barriers existed in *M. crassipes* × *M. yunnanensis* and *M. crassipes* × *M. foveolata*, whereas *M. crassipes* showed favorable cross-compatibility with *M. figo*. This high

Table 1. Hybridization processes and heterozygote embryogenesis of *Michelia yunnanensis* × *Michelia crassipes*.

Time after pollination	Hybridization processes and heterozygote embryogenesis
4 HAP	Pollen germination
5–8 HAP	Pollen tubes elongated into the style of <i>M. yunnanensis</i>
10 HAP	Pollen tubes merged into a bundle
12–24 HAP	Pollen tubes extended into the middle-low part of style
48 HAP	Pollen tubes entered in the ovary
3 DAP	Pollen tube bundle separated and reached each ovule
4 DAP	Double fertilization
5 DAP	4-cell proembryo
8 DAP	Multicellular proembryo
10 DAP	Globular embryo
14 DAP	Cordate embryo
20 DAP	Torpedo embryo
45 DAP	Mature embryo
85 DAP	Endothelium lignification
105 DAP	Black endothelium and mature seeds

DAP = days after pollination; HAP = hours after pollination.

Fig. 4. The germination of pollen grains and pollen tube growth of *Michelia yunnanensis* on the pistil of *M. crassipes* from 1 h after pollination (HAP) to 4 d after pollination (DAP) on the fluorescence microscope; yellow-green showed the pollen tubes. cd = callose deposition; (A–C) the pollen grains of *M. yunnanensis* could not germinate on the stigma of *M. crassipes* at the first 4 HAP; (D–F) a small number of pollen germinated on the stigma at 8 HAP, 10 HAP, 12 HAP, red arrow showed pollen grains; (G) massive pollens germinated at 24 HAP, red arrow showed pollen grains; (H) the pollen tubes merged into a bundle at 48th HAP, red arrow showed pollen grains; (I and J) the pollen tubes extended into the middle-low part of style at 3 DAP; (K) pollen tubes separated and failed in reaching each ovule completing double fertilization because of abnormal callose deposition; (L) red arrow showed callose deposition. Scale bar = 500 µm.

cross-compatibility in *M. crassipes* × *M. figo* was further verified and proved by the normal chromosome pairing and separation during meiosis (Zhang et al., 2011).

The sexual barriers hampering interspecific hybridization have been distinguished into pre- and postfertilization barriers (Stebbins, 1958). The details of pollen tube growth from stigma to ovule have been divided into four characteristic stages (Wilhelmi and Preuss, 1997). Any failure before the double fertilization belongs to the prefertilization barrier. Several reasons have been proposed to explain this phenomenon, including the pollen-wall proteins which served as recognition substance failing to recognize the stigma, hysteretic identification between the pollen and stigma, abnormal growth of pollen tubes, failure in recognition of pollen tubes to the style, pseudofertilization, and so on (Hogenboom and Mather, 1975; Knox et al., 1972). For the reciprocal cross, it was difficult for the stigma of *M. crassipes* to identify the pollen of *M. yunnanensis* at the first 6 HAP. Even though pollen grains of *M. yunnanensis* could germinate on the stigma of *M. crassipes*, they would fail to reach the ovule to complete the double fertilization because of abnormal callose deposition. Based on present observation, it could be inferred that incompatibility in the combination of *M. crassipes* × *M. yunnanensis*, which was in accordance with previous observation (Wang et al., 2003). The unilateral incompatibility of *M. crassipes* was resulted from the prefertilization barriers, the direct causes were hysteretic identification between the pollen and stigma and abnormal callose deposition.

Characteristic of heterozygote embryogenesis. The embryological characteristics of heterozygote of *M. yunnanensis* × *M. crassipes* observed in this study are consistent with other Magnoliaceae species (Fan et al., 1992; Xiao and Yuan, 2006). The embryo sac developed into a polygonum type, and endosperm development was the nuclear type. Without dormancy, the pollinated zygote underwent a series of tissue differentiation and formed 4-cell and multicellular proembryo, and then globular, cordate, torpedo, and mature embryo, which would develop into a mature seed together with endosperm. This kind of development process was also in accordance with the general embryological characteristics of dicotyledon (Boavida et al., 2005; Palser, 1975). Nevertheless, the earlier development stage including apical cell differentiation and basal cell differentiation was not detected in our study. The future collection of the unobserved data in this study will help us to refine the process of embryogenesis and clarify the heterozygote characteristics of interspecific hybridization of *Michelia*.

Guidance providing for interspecific hybridization of *Michelia*. As the existence of unilateral incompatibility on *M. crassipes*, some special treatments for overcoming prefertilization barriers may be advisable in the artificial hybridization when choosing

M. crassipes as maternal parent to cross with other paternal *Michelia* species (excepting for *M. figo* and *M. calcicola*). A range of pollination techniques such as bud pollination, use of mixed or mentor pollen, style or ovary manipulations, and chemical treatments might be applied to overcome prefertilization barriers (Pandey, 1977; Van Tuyl and De Jeu, 1997). The most suitable technique for *M. crassipes* has not been studied, which is deserved to be tested in future studies.

In addition, as the required time for the pollen tube growth from stigma to ovule varies considerably between species (Wilhelmi and Preuss, 1997), it would be meaningful to investigate the lasting time for double fertilization when confronting with the hybridization practices. In our study, as it took 4 d for the hybridization processes of *M. yunnanensis* × *M. crassipes* from artificial pollination to double fertilization, this duration would guide for the general bagging time and early identification of hybrids in future interspecific hybridization of *Michelia*.

Literature Cited

- Boavida, L.C., A.M. Vieira, J.D. Becker, and J.A. Feijo. 2005. Gametophyte interaction and sexual reproduction: How plants make a zygote. *Intl. J. Dev. Biol.* 49:615–632.
- Chen, D. and J. Zhao. 2005. Suitable cryosectioning technique in floral organs of plants. *J. Wuhan Bot. Res.* 23:285–290.
- De Nettancourt, D. 2013. Incompatibility in angiosperms. Springer Science & Business Media, New York, NY.
- Fan, R.W., J.G. Ye, Z.F. Yin, H.D. Gao, and L.X. You. 1992. Studies on seed development and embryogenesis in *Liriodendron chinense* (Hemsl.) Sarg. *Acta Bot. Sin.* 34:437.
- Fan, J.C., T.P. Yi, and Z.Z. Luo. 2009. A new variety of *Michelia linn.* (Magnoliaceae) in China. *J. Sichuan For. Sci. Technol.* 30:10001.
- Fu, X.Y. and W.B. Sun. 2006. In vitro culture of hybrid F₁ of *Michelia crassipes* Law (♀) × *Michelia calcicola* c. Y. Wu ex law et y. F. Wu. *Plant Physiol. Commun.* 42:461–464.
- Gong, X., Y.Z. Pan, and Z.Y. Yang. 2001. The cross-compatibility of Magnoliaceae. *Acta Bot. Yunnanica* 23:339–344.
- Gong, X., G.L. Zhang, and Y.Z. Pan. 2003a. A cytological study on two hybridized combinations of Magnoliaceae. *Acta Hort. Sin.* 30:615–617.
- Gong, X., G.L. Zhang, Y.Z. Pan, and Z.Y. Yang. 2003b. Three new varieties of *Michelia*. *Acta Hort. Sin.* 30:123.
- Han, C.Y., G.F. Lao, L. Ji, W.B. Sun, X.Y. Fu, and C.R. Li. 2014. ‘Chilongzhua’ and ‘Yanzhizui’: Two interspecific hybrid cultivars of *Michelia*. *HortScience* 49:96–97.
- Hogenboom, N.G. and K. Mather. 1975. Incompatibility and incongruity: Two different mechanisms for the non-functioning of intimate partner relationships. *Proc. R. Soc. Lond. B Biol. Sci.* 188:361–375.
- Ji, L. and W. Sun. 2011. Study on pollen germination of *Michelia crassipes*, *M. calcicola* and their F₁ hybrids. *Plant Sci. J.* 29:691–695.
- Jiang, J.M., X. Li, and N.R. Sheng. 1999. On the hybridization techniques of Magnoliaceous plants. *For. Res.* 12:214–217.
- Knox, R., R. Willing, and A.E. Ashford. 1972. Role of pollen-wall proteins as recognition substances in interspecific incompatibility in poplars. *Nature* 237:381–383.
- Law, Y.H. 2004. Magnolias of China. Beijing Science and Technology Press, Beijing, China.
- Lewis, D. 1958. Unilateral interspecific incompatibility in flowering plants. *Heredity* 12:233–256.
- Li, Y.J., Y.Z. Pan, and X. Gong. 2013. A new hybrid cultivar of *Michelia* ‘Yunxia’. *Acta Hort. Sin.* 40:1007–1009.
- Michel, M., T. Hillmann, and M. Muller. 1991. Cryosectioning of plant material frozen at high pressure. *J. Microsc.* 163:3–18.
- Palser, B.F. 1975. The bases of angiosperm phylogeny: Embryology. *Ann. Mo. Bot. Gard.* 621–646.
- Pan, L., Z.Y. Yang, G.L. Zhang, and X. Gong. 2008. Karyotypic studies on three hybrids and their parents in Magnoliaceae. *J. Trop. Subtrop. Bot.* 16:557–566.
- Pandey, K.K. 1977. Menton pollen: Possible role of wall-held pollen growth promoting substances in overcoming in intraspecific and interspecific incompatibility. *Genetica* 47:219–229.
- Risueno, M.C., P.S. Testillano, and P. González-Melendi. 1998. The use of cryomethods for plant biology studies, p. 3–4. In: H.A. Calderón-Benavides and M.J. Yacamán (eds.). *Electron microscopy*. Vol. 98. Inst. Physics, Philadelphia, PA.
- Shao, W.H., J.M. Jiang, and R.X. Dong. 2015a. A new *Michelia* cultivar ‘Mengzi’. *Acta Hort. Sin.* 42:1863–1864.
- Shao, W.H., J.M. Jiang, and R.X. Dong. 2016. A new *Michelia* cultivar ‘Mengxing’. *Acta Hort. Sin.* 43:1219–1220.
- Shao, W.H., J.M. Jiang, R.X. Dong, and Q.F. Luan. 2015b. A new variety, *Michelia* ‘Mengyuan’. *Scientia Sinica Sinica* 51:155.
- Stebbins, G.L. 1958. The inviability, weakness, and sterility of interspecific hybrids. *Adv. Genet.* 9:147–215.
- Van Tuyl, J.M. and M.J. De Jeu. 1997. Methods for overcoming interspecific crossing barriers, p. 273–292. In: *Pollen biotechnology for crop production and improvement*. Cambridge Univ. Press, Cambridge, NY.
- Wang, R.H., H. Jia, J.Z. Wang, and Z.X. Zhang. 2010. Flowering and pollination patterns of *Magnolia denudata* with emphasis on anatomical changes in ovule and seed development. *Flora* 205:259–265.
- Wang, Y.L., Y. Li, S.Z. Zhang, T.C. Cui, and X.L. Wu. 2003. The crossing result of Magnoliaceae. *J. Wuhan Bot. Res.* 21:508–514.
- Wang, F.G., Q.W. Zeng, R.Z. Zhou, and F.W. Xing. 2005. *Michelia rubriflora*, a new species of Magnoliaceae from Hainan island, China. *Pak. J. Bot.* 37:559–562.
- Wilhelmi, L.K. and D. Preuss. 1997. Blazing new trails (pollen tube guidance in flowering plants). *Plant Physiol.* 113:307.
- Xiao, D.X. and Z. Yuan. 2006. Embryogenesis and seed development in *Sinomanglietta glauca* (Magnoliaceae). *J. Plant Res.* 119:163–166.
- Zhang, X.C., Z.G. Li, S.L. Li, L. Lin, L.T. Yang, and Y.R. Li. 2008. Cryosectioning method for the observation of microtubule cytoskeleton in plant cells. *Guihaia* 28:164–166,178.
- Zhang, Z., L. Niu, X. Chen, X. Xu, and Z. Ru. 2012. Improvement of plant cryosection. *Front. Biol.* 7:374–377.
- Zhang, S.Z., Y.L. Wang, Z.C. He, and E. Ejder. 2011. Genome differentiation in Magonoliaceae as revealed from meiotic pairing in interspecific and intergeneric hybrids. *J. Syst. Evol.* 49:518–527.