Cytological Study of Tetraploid Species of *Magnolia* subgenus *Yulania* (Magnoliaceae)

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**Summary** Cytological studies were conducted on five tetraploid species plus two varieties of *Magnolia* subgenus *Yulania* and a hybrid between two of these species. Two different chromosome configurations in meiosis are observed in this article. The first group was typical for *M. acuminata*, *M. liliiflora* and their relatives which share several cytological characteristics including chromosome configurations and behavior in meiosis. The cytological evidence indicate autotetraploid origin for these species, despite the homologous chromosomes likely having changed their structures causing the formation of heteromorphic multivalents, chromosome bridges and fragments during meiosis. The second group of chromosome configuration was found in *M. cylindrica* and *M. concinna*. These species were characterized by very rare trivalents and tetravalents. These observations suggest these taxa as allotetraploids which may have been formed from hybridisation between hexaploid and diploid parents as deduced from trivalents observed at some rate. Multivalent formation and possible translocations and inversions of chromosomes in *M. liliiflora* influence its propagation by causing low fertility. *M. liliiflora* is probably a comparatively new species with an unstable chromosome constitution and is growing only in small populations at low elevation. It is vulnerable, and the species could already be extinct in nature. On the other hand, *M. acuminata* shows a more regular chromosome behavior and fewer abortion spores than *M. liliiflora*, and should be a more stable species with longer evolution time. Thus, the superior fertility of *M. acuminata* has enabled it to develop a stable and extensive distribution from NE to SE USA.


*Magnolia*, with a basic chromosome number of 19, is regarded as a paleopolyploid genus (Stebbins 1980). Two groups of this genus, subgenus *Magnolia* section *Theorhodon* Spach and subgenus *Yulania* (Spach) Reichenbach, have polyploid series based on tetraploid to hexaploid multiples of 19 chromosomes (Janaki Ammal 1952, Darlington and Wylie 1955, Chen et al. 1985). Law’s classification has been used in this study (Law et al. 2004).

Polyploidization in the speciation of *Magnolia* continues to attract the attention of taxonomists. The chromosome structure among *Magnolia* species varies little and the karyotypes of all the species remain constantly at type 2B (Stebbins 1971, Okada 1975, Biswas and Sharma 1984, Li et al. 1998). The chromosome behavior during meiosis is usually examined to determine the numerical and structural features of chromosomes. The configuration at metaphase I (MI) of meiosis is commonly showing the homologous degree between the different sets of chromosomes in polyploid plants (Jackson and Casey 1980). Further comparative analysis helps to explore the way of formation and evolution mode of the polyploidy. Very little work has been done to study the chromosome behavior during meiosis of Magnolias with the exception of a single study on the chromosome configuration of *M. denudata* Desr. (Li and He 2003) and a study on the genome differentiation between *Michelia* and subgenus *Yulania* based on hybrid chromosome configuration (Zhang et al. 2011).

Subgen. *Yulania* includes three taxonomically well-established tetraploid species of which two, *M. liliiflora* Desr. and *M. cylindrica* E. H. Wilson, occur in China and one, *M. acuminata* L., in North America. The latter includes two varieties, *M. acuminata* var. *acuminata* and *M. acuminata* var. *subcordata* (Spach) Dandy (Law et al. 1996).

*M. liliiflora* was described by Desrousseaux (de Lamarck 1792) with Kaempfer’s flower plates of plants in Japan used as the type. This species is frequently cultivated in gardens all over the world, but has never been recorded in the wild up to now. Existing herbarium samples do not verify an origin in the wild. The native range is suggested to be eastern China (Law et al. 1996).
It shows a notable variation in the number and size of tepals. The chromosome number is 2n=4x=76 (Whitaker 1933, Biswas and Sharma 1984, Li et al. 1998). M. liliiflora Desr. var. gracilis (Salisb.) Rehd. which may have originated in cultivation, has been described (Rehder 1916) and the history in cultivation has been discussed by Treseder (1978). We agree with the treatment of Frodin and Govaerts (1996) and Xia et al. (2008) to see M. liliiflora var. gracilis Rehd. as a synonym of M. liliiflora. M. polytepala Law & R. Z. Zhou & R. J. Zhang was described as a new species with 12–16 tepals from Mt. Wuyi of Fujian Province (Zhang et al. 2006). In our field explorations, we have found that M. polytepala has true bush habit with slim twigs and no ascending trunks even in individuals 20-years old. In contrast, older plants of M. liliiflora can have one or several trunks. A hexaploid of subgenus Yulania, M. sprengerii Pamp. also has rich variation in tepal number (Kang and Ejder 2011).

The tepal number is often a capricious character in subgenus Yulania as discussed by Treseder (1978). We agree with the treatment of Treseder (1978) to consider the species with 12–16 tepals from Mt. Wuyi of Fujian Province (Zhang et al. 2006) as a form of M. polytepala. In our study, both taxa above are treated as only different varieties of M. liliiflora from different distributions, but of horticultural interest. No studies about their chromosomes have been reported so far.

Magnolia cylindrica E. H. Wilson is distributed in southeast China at an altitude of 800–1900 m. The flowers have nine tepals, and the outer three are sepal-like with triangular shape. The chromosome number is 2n=76 (Parris et al. 2010). There is no earlier cytological information about meiosis. M. concinna Law & R. Z. Zhou was described as a new species with 12 tepals from Mt. Wuyi of Fujian Province (Law et al. 2004). Our long time observation of live plants, in cultivation as well as in the wild in Fujian Province, shows that M. concinna is similar to M. cylindrica in the shape of leaves and fruit, only that it has more tepals (9–12) than the latter. Since the occasional appearance of specimens with additional tepals is a general phenomenon in Magnolia, it should be submersed into M. cylindrica (Xia et al. 2008, Kang and Ejder 2011). We treat this taxon as an ecotype of M. cylindrica in this study.

Magnolia acuminata is distributed in eastern North America from New York state and southern Ontario, south to the Florida panhandle and west to Arkansas and Louisiana. The only species in subgenus Yulania with yellow tinted flowers, M. acuminata typically produces blooms that are fairly variable in tepal size and color (from green, yellow-green to yellow) while there is little or no pubescence on the young twigs and abaxial sides of leaves (Callaway 1994). Its variety, M. acuminata var. subcordata (Spach) Dandy, which is restricted to SE US, has more consistently yellow flowers while its young twigs and leaf-backs are covered with dense pubescence. It is also a smaller tree, sometimes even shrub-like, than the much larger-growing M. acuminata var. acuminata.

M. ×brooklynensis Kalmbacher, the hybrid between M. acuminata and M. liliiflora, exceeds M. liliiflora in fertility and hardiness. The cytology of the clone M. ×brooklynensis ‘Woodsman’ was studied in this investigation in order to confirm the cytological relationship of its parents.

This article presents a comparison of the chromosome behavior in pollen mother cells (PMCs) of these five species, plus two varieties and one hybrid, all belonging to Magnolia subgenus Yulania. The study aims to provide cytological evidence to unravel the origin of these tetraploids and the relationship among them. In order to study the intra-species variation on chromosome level, several individuals of different provenances have been observed.

Materials and methods

Seven taxa of Magnolia subgenus Yulania were studied including M. liliiflora Desr. (four samples from different sources), M. liliiflora var. gracilis (Salisb.) Rehd. (one sample), M. polytepala Law et R. Z. Zhou et R. J. Zhang (one sample), M. acuminata L. (two samples with different morphological characters), M. acuminata var. subcordata (Spach) Dandy (one sample), M. cylindrica E. H. Wilson (four samples with different morphological characters from different sources), M. concinna Law et R. Z. Zhou (one sample) and in addition a hybrid M. acuminata×M. liliiflora (one sample) (Table 1).

The living material and voucher specimens are grown respectively deposited at Wuhan Botanical Garden (HIB), Shenzhen Fairylake Botanical Garden (SZG), and Xi’an Botanical Garden (XBG). The M. cylindrica, M. cylindrica ‘Pegasus’ and M. cylindrica ‘Krossa’ used originated from Huangshan (Anhui Prov.) or Lushan (Jiangxi Prov.) in China and have been cultivated in Western gardens for a long time as M. cylindrica. The specimens used are growing in the gardens of Dr. Finnck (FG) or Dr. Ejder (EG) in Sweden (Table 1).

Preparation of somatic cell chromosomes: Leaf apical meristem was pre-treated in a mixed solution of 0.1% colchicine and 0.002 mol/L 8-hydroxyquinoline for 3 h before fixation in Carnoy’s fixative (glacial acetic acid: ethanol=1:3) for 1 h before squashing. It was dyed and then observed with an optical microscope.

Preparation for PCMs: Flower buds were taken when the PCMs were in the meiotic phase during early spring. After removal of the bud bracts and tepals, the immature anthers were fixed in Carnoy’s fixative for 8–24 h. After cellulase and pectinase digestion of cell walls, a flame-drying protocol was followed to make chromosome preparations (Zhu 1982). These chromosomes were stained by the Giemsa method. All meiotic stages were studied and photographed using a Zeiss Axioplan 2 imaging microscope equipped with an AxioCam digital camera.
either chromosome fragments or micronuclei. Of the 127 chromosomes or micronuclei.

arrays of tetrads. Some cells in MII had either lagging developed into isobilateral, decussate, linear to T-shaped either perpendicular or parallel to each other. The cells II (MII) were lined on the equatorial plates which were number in two daughter cells (Fig. 1F) (Table 3). The chromosome fragmentation and different chromosome cells displayed abnormalities like chromosome bridges, 101 randomly selected PMCs in AI, 12.9% of the -2). The normal division figure of anaphase I (AI) exhib =2n

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tromes. Because of the great number and length of the chromonemata and the multivalent pairing, the chromo-
nesult of contraction and pairing of homologous chromo-
somes. The PMCs of the four specimens of different prove-

Results

Magnolia liliiflora Desr.

The following report is mainly based on observation of the sample from Wuhou Temple of Mian County in Shaanxi Province.

The PMCs of the four specimens of different provenances (Table 1) show similar chromosome performance during meiosis. The chromatin in prophase I contracted into chromonemata which became thicker and shorter as a result of contraction and pairing of homologous chromosomes. Because of the great number and length of the chromonemata and the multivalent pairing, the chromonemata were entangled with each other and could hardly be identified during pachytene (Fig. 1A) and in diplotene (Fig. 1B). A number of multivalents can be observed in every PMC in the diakinesis stage (Fig. 1D). Multiva-
tents were observed in early MI, but most of them will gradually resolve into bivalents in late MI. Bivalents dominated though ring or chain tetravalents and one het-

ero-hexavalent also coexisted in early MI (Fig. 1C, E).

Among the 23 PMCs studied, the average chromosome configuration was $2n=34.83\text{II}+1.39\text{IV}+0.13\text{VI}$ (Table 2). The normal division figure of anaphase I (AI) exhibits clearly the chromosome number as $n=2x=38$. Among 101 randomly selected PMCs in AI, 12.9% of the cells displayed abnormalities like chromosome bridges, chromosome fragmentation and different chromosome number in two daughter cells (Fig. 1F) (Table 3). The chromosomes of the two daughter cells in metaphase II (MII) were lined on the equatorial plates which were either perpendicular or parallel to each other. The cells developed into isobilateral, decussate, linear to T-shaped arrays of tetrads. Some cells in MII had either lagging chromosomes or micronuclei.

The chromosome number in anaphase II (AII) is $n=2x=38$ (Fig. 1G). 15.6% of 45 cells examined had either chromosome fragments or micronuclei. Of the 127 pollen grains observed by random selection, 65 were empty, indicating that more than half of the grains were sterile. The abnormal chromosome behavior in meiosis and huge proportion of infertile pollen is a consequence of multivalent formation at MI and irregular chromosome segregation at AI and AII.

The chromosome behavior of PMCs and the arranging patterns of microspore tetrads of $M. liliiflora$ from Hubei Province was similar to those described above. Multi-
tavents could be observed both in diakinesis and early MI. Up to one ring hexavalent and five tetravalents (of which 4 were hetero-tetravalents) could be observed in early MI (Fig. 1I). Chromosome bridges and fragments were found in about 10% of the cells in AI and AII.

The PMCs of the sample from Mount Micang of Shaanxi Province, suspected to be a wild individual, exhibited the same features during meiosis, with more ring bivalents and tetravalents dominating in diakinesis. Up to one hetero-hexavalent or five tetravalents (of which four are hetero-type) were observed in early MI (Fig. 1J). In AI, chromosome bridges and fragments were seen in a few PMCs.

The samples from Hangzhou Botanical Garden of Zhejiang Province demonstrated the same features as the other three: five tetravalents were found at most in one PMC (Fig. 1K).

Magnolia liliiflora var. gracilis Rehd.

The chromosome number was found to be $2n=4x=76$. Bivalents dominated, accompanied by hetero-tetravalents and hexavalents in early MI (Fig. 1L). Four tetravalents were found in one PMC at most (Fig. 2A). The chromo-
some configuration is $2n=34.58\text{II}+1.47\text{IV}+0.16\text{VI}$. The behavior of chromosomes at AI, MII and AII is normal.

According to our study of the samples, the plant mor-

Table 1. Sources of materials used in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>Voucher</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. liliiflora</em> Desr.</td>
<td>Wuhou Temple, Mianxian, Shaanxi</td>
<td>SZG</td>
</tr>
<tr>
<td><em>M. liliiflora</em> Desr.</td>
<td>*Wuhan Botanical Garden, Wuhan, Hubei</td>
<td>HIB</td>
</tr>
<tr>
<td><em>M. liliiflora</em> Desr.</td>
<td>*Mt. Micang, Hanzhong, Shaanxi</td>
<td>XBG</td>
</tr>
<tr>
<td>M. liliiflora var. gracilis (Salisb.) Rehd.</td>
<td>Hangzhou Botanical Garden, Hangzhou, Zhejiang</td>
<td>SZG</td>
</tr>
<tr>
<td>M. polytetrapala Law et R. Z. Zhou</td>
<td>Xi’an Botanical Garden, Shaanxi (Huangshan origin)</td>
<td>XBG</td>
</tr>
<tr>
<td>M. cylindrica E. H. Wilson</td>
<td>Flinck Garden, Sweden (Huangshan origin)</td>
<td>FG</td>
</tr>
<tr>
<td>M. cylindrica ‘Krossa’</td>
<td>Ejder Garden, Sweden</td>
<td>EG</td>
</tr>
<tr>
<td>M. concinna Law et R. Z. Zhou</td>
<td>Flinck Garden, Sweden</td>
<td>FG</td>
</tr>
<tr>
<td>M. acuminata L. (Kenneth’s Delight)</td>
<td>Mt. Wuyi, Fujian</td>
<td>XBG</td>
</tr>
<tr>
<td>M. acuminata L. (‘North Gold’)</td>
<td>Flinck Garden, Sweden (US origin)</td>
<td>FG</td>
</tr>
<tr>
<td>M. acuminata L. var. subcordata (Spach) Dandy (‘Miss Honeybee’)</td>
<td>Flinck Garden, Sweden (Wyoming Co, NY, USA)</td>
<td>FG</td>
</tr>
<tr>
<td>M. acuminata x M. liliiflora (‘Woodsman’)</td>
<td>Flinck Garden, Sweden (US origin)</td>
<td>FG</td>
</tr>
</tbody>
</table>

*Suspected to be wild clone.
These facts support the view above that *M. liliiflora* var. *gracilis* should be a synonym of *M. liliiflora* and not given varietal status.

Magnolia polytepala *Law* et *Z. Zhou* ex. *R. J. Zhang*

The chromosome number is found to be $2n=76$. The same features concerning both the chromosome behavior in PMCs and the tetrad type were observed as in *M. liliiflora* and *M. liliiflora* var. *gracilis*. Bivalents prevailed, while four ring or chain tetravalents or one hetero-hexavalent existed in some PMCs (Fig. 2B–D). The chromosome configuration is $2n=0.04I+34.32II+0.04I$.
II+1.41IV+0.27VI (Table 2). The behavior of chromosomes at AI, MII and AII is basically normal. Its chromosome configuration and performance during meiosis are totally the same as those of M. liliiflora. These facts support the view above not to recognize M. liliiflora as a separate species. Magnolia acuminata L.

M. acuminata 'Kenneth’s Delight' and M. acuminata 'North Gold' are used in this study (Table 1). They show the same chromosome behavior during meiosis. M. acuminata is a tetraploid, 2n=4x=76, in agreement with previous studies (Whitaker 1933).

The chromosome behavior in PMCs is similar to that of M. liliiflora. Tetravalents can be clearly observed in the zygote stage (Fig. 3A). In diakinesis, ring bivalents and tetravalents are frequently seen (Fig. 3B and C), but only bivalents are seen in later MI. Bivalents dominate during MI, though one to four ring or chain tetravalents and one hetero-hexavalents were observed (Fig. 3D and E). The chromosome configuration of 56 PMCs at MI was 2n=33.84II+1.63IV+0.30VI (Table 2). Five tetravalents at most could be observed within one PMC (Fig. 3D). Interlocked bivalents were found, which might give rise to inversions or translocations and lead to the appearance of chromosome fragments in AI.

The figure of AI and AII show that the chromosome appearance of chromosome fragments in AI. The number of bivalents appearing in one cell. Same principle in the rest of the table. Number of bivalents appearing in one cell, ranging from 28–38. Same principle in the rest of the table. 1Mean number of bivalents appearing in one cell. Same principle in the rest of the table. 2Number of bivalents appearing in one cell, ranging from 28–38. Same principle in the rest of the table.

Magnolia acuminata L.

M. acuminata L. var. subcordata (Spach) Dandy

The chromosome behavior is similar to M. acuminata. During prophase I, especially at diplotene and diakinesis, several tetravalents and one hexavalent could be observed (Fig. 3I). Up to seven tetravalents in one PMC existed during early MI of which 4 were of hetero-type (Fig. 3J). The chromosome configuration is 2n=34.40II+1.77IV+0.14VI based on 66 PMCs at MI (Table 2). 14% of 105 PMCs during AI had lagging chromosomes, chromosome bridges, and fragments. Similar abnormal phenomena were also found in AII.

Magnolia acuminata×M. liliiflora (M.×brooklynensis 'Woodman')

The chromosome configuration is nearly the same as that of its parents. And also similar to its parents, one chain hexavalent (Fig. 3K) was found in addition to several tetravalents during diakinesis. At MI, seven tetravalents at most were observed in one PMC, of which four were hetero-type (Fig. 3L). A chain hexavalent, possibly involving translocation chromosomes, was also recorded in this hybrid.

This means the four chromosome sets of the genomes of M. liliiflora and M. acuminata have not only the same type of polypliod origin, but also homologous chromosomes. But there are still some differences in the chromosome structures such as more ring bivalents at diakinesis in M. acuminata than in M. liliiflora.
Magnolia cylindrica *E. H. Wilson*

The chromosome behavior of four specimens of *M. cylindrica* of different origins are almost the same. The following results are mainly based on our observation of the sample in Xi’an Botanical Garden, which is from Mount Huangshan in Anhui, where the type specimen was collected (Rehder and Wilson 1927).

The chromosome number is $2n=4x=76$ in a number of provenances and clones, in agreement with previous studies (Parris et al. 2010).

Both the chromosome behavior and the tetrad type differ very much from those of *M. liliiflora*. The homolo-
hensive chromosomes are paired to bivalents, dispersing along the edge of PMCs. Chromonemata without synopsis were partly entangled in the middle of the PMCs. This pattern indicates non-simultaneous chromosome synopsis in some PMCs. It was observed that the ends of some homologous chromosomes did not pair in pachytene (Fig. 2E). Bivalents line up along the equatorial plate normally in the early phase of MI, while some of them separate early in late MI (Fig. 2H). Heteropyknotic chromosomes were observed in a few PMCs of MI, which leads to abortion microspores. A solitary ring bivalent and 37 rod bivalents are observed in PMCs of
The chromosome configuration showed more regular pairing patterns than those of *M. liliiflora* and *M. acuminata*: \(2n=2.00+35.50I+0.60II+0.30IV\) (Table 2). Though bivalents were the dominating type, one heterotetrapetal could occasionally be observed, and no hexavalent ever existed (Fig. 2F, G). At the AI and AII stages, abnormal behaviors, like lagging chromosomes, bridges and fragments, were observed in a rate of 14% in AI and 20% in AII (Table 3). Few abortion tetrads and pollen grains were seen.

**Magnolia concinna Law et R. Z. Zhou**

The chromosome number of the somatic cells was found to be \(2n=76\), and its chromosome configuration in meiosis is similar to *M. cylindrica*, \(2n=37.66I+0.17IV\) (Table 2). Though bivalents are still the dominating type, tetravalents are occasionally observed (Fig. 2I and J). At AI and AII, lagging chromosomes existed in about 50% of the cells (Fig. 2K and L). These facts support our view above not to recognize *M. concinna* as a separate species but treat it as an ecotype of *M. cylindrica*.

**Discussion**

**Cytological characters of tetraploid species in subgenus Yulania**

Two types of chromosome configurations during meiosis were observed in the tetraploids species of *Magnolia* subgenus *Yulania*. The first type includes five taxa, *M. liliiflora*, *M. liliiflora* var. *gracilis*, *M. polyte-pala*, *M. acuminata* and *M. acuminata* var. *subcordata* (called the "*M. liliiflora* group" below). The cytological characteristics can be summarized as follows: Bivalents were found to dominate while some tetravalents and hexavalents existed at early MI, but multivalents were seldom found in late MI. Except for seven tetravalents found in one PMC of *M. acuminata* var. *subcordata*, all the species have five tetravalents at most, part of which are asymmetrical hetero-tetravalents; the very similar chromosome configurations in meiosis show the stable nature in the chromosome behavior of this first group of species; the existence of ring hexavalents reveals the possible homoeology among 19 chromosomes of the genome originating possibly partly from similar genomes; they are all translocation heterozygotes, perhaps since a limited number of clones are investigated.

As a comparison, *Magnolia kobus* ‘Norman Gould’ is an artificial autotetraploid created by colchicine treatment (Janaki Ammal 1952). The chromosome configuration at MI is \(2n=5.30+28.54I+2.06II+1.86IV\). A few tetravalents can be observed in diakinesis and early MI (Zhang, S. Z., unpublished results). Since homologous chromosomes are often given priority in PMCs, the rate of the formation of multivalent pairing will be reduced a lot in nature in comparison to theory. The majority of the chromosomes at MI appear as bivalents and the limited number of multivalents might reflect the incomplete cytological diploidization of these tetraploids, irrespective of their exact mode of origin.

Based on all the results above, the origin mode of the species of the *M. liliiflora* group, are inferred as likely autotetraploids, but the homologous chromosomes among the four chromosome sets in the tetraploids have undergone obvious changes in their structures, as heteromorphic multivalents, chromosome bridges and fragments appeared during meiosis. The hetero- or homo- hexavalents and tetravalents suggest these taxa as translocation heterozygotes. The chromosome bridges and fragments in AI point to the existence of inversion chromosomes.

The second kind of chromosome configuration was found in the taxa of the second group comprising *M. cylindrica* and *M. concinna* (called the "*M. cylindrica" group" below), whose cytology differs greatly from the taxa of the first group described above. The latter group shows dominating bivalents with very rare tetravalents and trivalents; synthesis chromosomes pairing loosely in pachytene and MI are observed and a great quantity of lagging chromosomes in AI and AII. The chromosome configuration indicates that the two taxa are possibly allytetraploids, most likely hybrids between hexaploid and diploid parents, as deduced from trivalents observed at some rate, loosening synthesis chromosomes in pachytene and early separating chromosomes.

The possible hybrid origin of these tetraploids may contribute to a faster process of cytological diploidization, and thus fewer multivalents in meiosis. Genetic regulation of meiosis in tetraploids should also be considered (Cifuentes et al. 2010).

Since the current results are only based on cytology, the interpretation is as above until further evidence is made available by the application of alternative methods such as genomic in situ hybridization (GISH).

**Translocation heterozygotes**

Except for the *M. cylindrica* group, one or very rarely two ring hexavalents were seen in the PMCs of the *M. liliiflora* group. More pairing chromosomes were observed than expected, indicating translocation between non-homologous chromosomes. Nevertheless, no homozygote plants were found in the study. This begs the question whether the frequency of homozygotes is very low due to translocation heterozygotes being more competitive. This argument is consistent with reported evidence obtained from diploid *Oenothera biennis* L. and tetraploid *Haworthia reinwardtii* var. *chalumensis* G. G. Smith. In the latter taxon, the frequency of translocations can exceed 99% of the individuals per population (Brandham 1974, Levy and Levin 1975). These patterns are rather similar to our observations on *M. liliiflora*.

Moreover, we noticed that multivalents were observed
in almost all PMC at diakinesis and early MI in the tetraploids investigated. These multivalents were kept separated and turned into normal bivalents in MI because of no chiasmata and no matching of the chromosomal termini. The lack of cross over held the chromosomes together in ring multivalents, which in turn is helpful in preserving the translocation structure of the chromosomes. It is reported that heterozygote individuals in Oenothera can have high genetical variation and produce apparent variations without hybridization (Brandham 1974). This may explain the wide degree of polymorphism (i.e., growth habit, flower color and shape) in both M. liliiflora and M. acuminata.

**Speciation of M. liliiflora**

Autopolyploidy can be caused by unreduced, diploid gamete production, which can be as high as 4% in some angiosperm species (Ballington and Galletta 1976, Maceira et al. 1992). A good example is M. pseudokobus Abe & Akasawa, which is a spontaneous triploid cytotype of wild Japanese origin (Ueda 1986). Also, a triploid Michelia was obtained through artificial pollination (Wang et al. 2006). The fusing of unreduced gametes may be the origin of the tetraploid M. liliiflora. In addition to production of unreduced gametes, autopolyploids can also arise as a result of somatic chromosome duplication.

Reduced fertility of nascent polyploids is mostly due to meiotic irregularities. Complicated configurations, caused by polyploidization and inversions or translocations, prevent it from stabilizing. Translocation chromosomes also severely affect the fertility of microspores of M. liliiflora. However, the absence of some chromosome segments can be compensated or substituted by other relevant normal chromosomes in polyploids. Even though there were no more abnormal divisions observed at the microspore mother cell meiosis stage than in the case of M. acuminata, M. acuminata var. subcordata and M. cylindrica, the most serious abortion of M. liliiflora should happen at the mononuclear and late binuclear pollen stages. Only 49% normal pollen can be observed finally, much lower than for other species (Table 3). The same phenomena were reported for autotetraploid rice pollen (Dai et al. 2006). It has been inferred that an insufficiency of nutrition is the reason in addition to the abnormal division of chromosome at meiosis (Peng et al. 2003, Wang and Zhang 2008).

The great number of pistils and stamens is another means of making up for the high proportion of infertile pollen and ovules. M. liliiflora may still exist as small and isolated populations in low altitude forests (600–800 m). But the impaired fertility would be expected to limit expansion of populations of M. liliiflora. In addition, forest habitat destruction and consistent human digging of plants likely increased the threat of local extinction. This species seems to be naturally restricted to low and middle altitude which has made the human impact even more pronounced than for several other Magnolia species. Thus populations were rapidly destroyed and may have been extinct already in the wild before it reached a stabilized genome (Parisod et al. 2010).

M. liliiflora, which is perhaps the earliest ornamental plant in the Magnoliaceae (with the possible exception of M. denudata), has been cultivated for more than 2500 years (Law et al. 1990). During this time its structural variations in the chromosomes have been preserved due to substantial vegetative propagation, such as artificial cutting, grafting and plant division. M. liliiflora should be seen as species of a late speciation and further evolution with a complicated genome restructuring. Genomic stabilization has not been effective during this phase of decline. The possibility of repeated polyploidisation in different populations must also be considered (Soltis and Soltis 2000).

In the results from DNA sequencing studies, no existing diploids, such as M. kobus D.C. or M. biondii Pamp., are close to M. liliiflora. But it has a very close relationship with M. denudata Desr. and M. cylindrica (Qiu et al. 1995, Azuma et al. 1999, Kim et al. 2001, Wang et al. 2002, Nie et al. 2008), and it should be noted that both M. denudata and M. liliiflora are polyploids. Maybe they share the same extinct diploid parent from earlier periods of evolution. A polyploid offspring could have found a niche in the competition with the diploid parent at first. But during colder climatic periods, the diploid parent may not have been able to adapt to the cold as well as the polyploid individuals (=polyploid advantage) and thus became extinct. Tetraploid and hexaploid populations may show a higher survival probability and disperse in high altitude mountains (Parisod et al. 2010, te Beest et al. 2012). Similar conditions have been discussed for M. sprengeri by Kang and Ejder (2011).

**Relationship of M. liliiflora and M. acuminata**

Subgenus Yulania has a disjunct distribution in eastern Asia and North America. Studies of fossils, palynology and geography show that its present geographical distribution was formed by the end of the Miocene (Hebda and Irving 2004). M. acuminata is distributed disjunctively from other species of subgenus Yulania, but have homologous chromosomes in its four sets of genomes and likely originated in the same way as M. liliiflora, an autotetraploid. The chromosome configurations in meiosis of these two species show a remarkable level of agreement in the nature of the chromosome behavior. M. liliiflora and M. acuminata originated from different diploid progenitors or, alternatively, they could have emerged from the same precursor diploid parent, but then their subsequent allopatric separation beginning in the Oligocene (Azuma et al. 2011, Hebda and Irving 2004, Nie et al. 2008) would account for their contrasting morphologies.
Although both seem to be autotetraploids, there are still some differences in the chromosome structures such as more ring bivalents at diakinesis in *M. acuminata* than in *M. liliiflora*. The tectate-perforate pollen in the exine structure of *M. acuminata* (Fig. 3H) is also further in evolution than the tectate-imperforate pollen of *M. liliiflora* (Fig. 1H) (Walker 1974). *M. acuminata* has a better fertility which enables it to be widely distributed, from the NE to the SE of the USA, although never abundant (Smith 1990). The fast growth makes it competitive with other trees, without the risk of getting out-competed like *M. liliiflora*. Although autotetraploids have reproductive problems because of the abnormal meiosis, fertility can be improved after subsequent genome evolution (Parisod et al. 2010). *M. acuminata* should have undergone a longer evolution period and be more stable in chromosome structure than *M. liliiflora*.

Compared to *M. acuminata*, the polyploidisation and speciation of *M. liliiflora* may be a relatively recently event. Nie et al. (2008) put forward that *M. acuminata* is the basal group of subgenus *Yulania* and *Michelia* based on the nuclear sequence data. The present study shows that *M. acuminata* is more closely related to *M. liliiflora* and other species of subgenus *Yulania* than it is to *Michelia* (Zhang et al. 2011), and this is supported by results of cpDNA studies (Qiu et al. 1995, Azuma et al. 1999, Kim et al. 2001, Wang et al. 2002).

**Speciation of M. cylindrica**

Allopolyploids are likely to have more capacity for novel evolution than autopolyploids (Madlung and Wendel 2013). In fact, large wild populations of *M. cylindrica* can often be found in high altitude mountains (900–1900 m) of eastern China. It is most likely a hybrid between hexaploid and diploid parents, deduced from trivalents observed in some rate. The fixed heterozygosity of allopolyploids (Soltis and Soltis 2000) may be responsible for persistent vitality and hardiness in natural populations of *M. cylindrica* in excess of what is required as an adaptation to the climate as shown by climate data and accompanying flora. Without giving any theoretical background, it has been noted as surprising and remarkable (Treseder 1978, Callaway 1994) that *M. cylindrica* is much more winter hardy (down to about −30°C) in cultivation in northern localities in Europe and USA than would be expected when considering its southerly natural distribution, 26°–30° N.

Horizontal gene transfer via hybridization may result in the exchange of DNA among species belonging to different lineages. Phylogenetic trees for many Magnoliaceae species have been constructed based on sequencings of plastid or nuclear DNA. In Magnoliaceae, as in other early angiosperms, it is generally considered that chloroplast DNA is inherited maternally (Corriveau and Coleman 1988, Tobe et al. 1993) in contrast to the contributions to the nuclear DNA from both gametes. Thus it is indeed striking to find that *M. cylindrica* shows a discordance between nuclear and cytoplasmic data. The most recent cpDNA-based molecular phylogeny shows *M. cylindrica* as most closely related to *M. denudata* and *M. liliiflora* (Azuma et al. 2011). The most recent corresponding phylogeny based on nuclear DNA (Nie et al. 2008) has *M. cylindrica* as most closely related to a *M. sprengeri* clone and rather far removed from *M. denudata*. The diploids *M. kobus*, *M. salicifolia* and *M. zenii* are all closer to *M. sprengeri* than *M. denudata* is (maximum likelihood tree only available). The position of *M. cylindrica* has not been commented on in these two investigations, but while these sequencings are not ideal for high resolution at species level, they may give some support to our suggested speciation process for *M. cylindrica*. It should be noted that *M. denudata* and *M. cylindrica* have several overlapping populations in eastern China, such as in Zhejiang and Jiangxi Provinces. The hexaploid parent could be *M. denudata*, and the diploid parent might have been extinct during evolution.

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**References**


