

## New relationships defined for Magnolia Species of Section Rytidospermum

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The Rytidospermum Magnolias have always been an intensely interesting group, not only for their large flowers and enormous whorled leaves, but because several species occur in both eastern Asia and eastern North America. This intercontinental trait is shared only with section Tulipastrum, but in that case the two species involved, *M. acuminata* and *M. liliiflora* (*M. quinquepeta* Buc'hoz), share few characteristics beyond the same chromosome number and the presence of reduced outer tepals. So it is the Rytidospermum species, with their many similarities, that truly arouse our curiosity. Did today's species evolve from a common ancestor? If so, how and when did its descendants cross the Pacific Ocean? Which one of the North American species is the most closely related to its Asian counterpart(s)? Using modern molecular biology, researchers Yin-Long Qiu, Clifford Parks, and Mark Chase analyzed the chloroplast DNA (cpDNA is the part of the DNA strand that is responsible for photosynthesis) of all section Rytidospermum species. By comparing the changes in the cpDNA of the various species, they were able to quantify the amount of evolutionary change (molecular divergence) that had taken place between them. Their results were published in two separate articles of the *American Journal of Botany* in 1995. The objective of this paper is to provide an overview and interpretation of this work, to summarize the relevant findings, and to suggest appropriate changes in the current series divisions for section Rytidospermum.

The study team (Qiu *et al.*) used three different laboratory techniques to assess the divergence among *Magnolia officinalis* var. *biloba*, *M. hypoleuca*, *M. tripetala*, *M. fraseri*, and *M. macrophylla*. [These analytical methods are extremely

complex, thus, for additional details on the materials and methods please refer to the study teams' original papers (Qiu, Parks, and Chase, 1995; Qiu, Chase, Parks, 1995).] The first method, cpDNA restriction site analysis, randomly samples changes (between all combinations of pairs of species) over the entire chloroplast gene, which amounts to 424 sites or about 2% of the entire gene. The analysis accounts for the number of site changes encountered, then calculates the cpDNA sequence divergence (as % sequence divergence) between all species pairs. The results are shown in Table 1:

This analysis clearly shows that *M. tripetala* from eastern North America has diverged far less from the Asian species *M. hypoleuca* and *M. officinalis* var. *biloba* than it has from other North American species. It also indicates that the other North

Table 1:

Species Pair	% Divergence
hypoleuca vs. biloba	0.04%
tripetala vs. hypoleuca	0.08%
tripetala vs. biloba	0.08%
macrophylla vs. hypoleuca	0.38%
fraseri vs. tripetala	0.41%
fraseri vs. hypoleuca	0.42%
fraseri vs. biloba	0.42%
macrophylla vs. tripetala	0.42%
macrophylla vs. biloba	0.42%
fraseri vs. macrophylla	0.45%

American species have diverged just as much from each other (including *M. tripetala*) as they have from the two Asian species.

The study team used a second method, allozyme electrophoresis, to examine genetic variation of enzymecoding genes. This analysis results in the calculation of a parameter called Nei's unbiased genetic identity for each of the species pairings. The numbers are from zero to one, with one being a



perfect genetic match. According to Clifford Parks (pers. comm.) as a rule of thumb, readings >0.90 suggest populations of the same species while readings <0.67 indicate distinctly different species. The results are shown in Table 2:

This second method almost mirrors the results of the restriction site analysis – very strong evidence for the *M.*

Table 2:

Species Pair	Nei's Genetic ID
hypoleuca vs. biloba	0.897
tripetala vs. hypoleuca	0.809
tripetala vs. biloba	0.712
fraseri vs. hypoleuca	0.444
macrophylla vs. tripetala	0.423
fraseri vs. biloba	0.419
macrophylla vs. hypoleuca	0.365
fraseri vs. tripetala	0.321
macrophylla vs. biloba	0.292
fraseri vs. macrophylla	0.234

*tripetala* connection with the Asian species, and relatively weak relationships between the rest of the species. Though not shown in the table, it should also be noted that Nei's genetic identity for infraspecific comparisons was nearly 1.000, as would be expected [values ranged from 0.993 for *M. hypoleuca* vs. *M. hypoleuca* to 0.932 for *M. macrophylla* vs. *M. macrophylla*]. It is interesting to note that in both analyses the relationship between *M. fraseri* and *M. macrophylla* is the most distant of any of the pairs. Ironically, some texts on North American trees refer to these two species as closely related on account of their similar auriculate leaves!

Lastly, the study team compared *M. tripetala*, *M. macrophylla* and *M. hypoleuca* to each other by sequencing a specific portion of the chloroplast gene called *rbcl*. This analysis involves comparing the 1432 base pairs of the *rbcl* gene for each pair of species in the analysis, which in this case is three (*M. macrophylla* vs. *M. hypoleuca*, *M. macrophylla*

divergence between *M. tripetala* and *M. hypoleuca* for that portion of the DNA strand.

Qiu, Chase, and Parks feel that since the results from all three methods have yielded the "same pattern of divergence," that they can be considered reliable for determining divergence between those *Magnolia* species. They emphasize that the "molecular divergence between *M. tripetala* and its

Table 3:

Species Pair	% Divergence
tripetala vs. hypoleuca	0.000%
tripetala vs. macrophylla	0.140%
hypoleuca vs. macrophylla	0.140%

Asian sister taxa, *M. officinalis* var. *biloba* and *M. hypoleuca*, is extremely low—the lowest divergence ever reported for any eastern Asia—eastern North America disjunct taxa." For example, the sequence divergence over the entire chloroplast gene (cpDNA) between *Liriodendron tulipifera* and *L. chinense* (Qiu, *et al.* 1993; Parks and Wendel, 1990) was found to be 1.240% (as compared with 0.083% between *M. hypoleuca* and *M. tripetala*), which is somewhat remarkable in that for quite some time many taxonomists considered both *Liriodendron* taxa to be varieties of the same species.

The study team provided speculation as to how and when *M. tripetala* and its sister species became separated from their common ancestor. One of the hypotheses is that the common ancestor could have migrated between the continents via the Bering land bridge during one of earth's warm periods in the middle Miocene (17 to 15 million years before present) or early Pliocene (6 to 5 mybp). They suggested that more examination of fossil evidence may be needed. Many of you may recall that cpDNA was sequenced from fossil *Magnolia* leaves from Clarkia, Idaho recently (Golenberg, *et al.*, 1990). I visited Clarkia in 1991 (Figlar, 1993) and studied many fossil *Magnolia* leaves found at the site. A few of those leaves strongly resemble leaves from modern section *Rytidospermum* magnolias. The one pictured here (Figure 1) has strikingly



similar leaf venation patterns to present day *M. hypoleuca* and *M. tripetala*.

In their second paper, the research team expanded their phylogenetic study using restriction site analysis to include *M. officinalis* var. *officinalis*, *M. rostrata*, *M. macrophylla* var. *ashei*, *M. macrophylla* var. *dealbata*, *M. fraseri* var. *pyramidata*, as well as many other magnoliaceous taxa in the species pairings. One result was an expanded sister relationship between *M. tripetala* and its Asian species to include *M. officinalis* var. *officinalis* and *M. rostrata* in the sister group. Other findings in their analysis include:

1. Though *M. officinalis* var. *officinalis* and *M. officinalis* var. *biloba* are closely related (part of the sistergroup), they are separated by four restriction site changes which is more than the one restriction site change between *M. officinalis* var. *officinalis* and *M. rostrata*. This suggests that "full species status for *M. o.* var. *biloba* is justifiable," but the team recommends a detailed study of wild populations of all four Asian taxa before any decision is made.

2. Only one restriction site change was found between *M. macrophylla* var. *dealbata* and *M. macrophylla* var. *ashei* and no change separating these two from *M. macrophylla* var. *macrophylla*. The study team agrees with Dorothy Callaway (Johnson, 1989) and rejects species status for the former two varieties.

3. Similar to number 2 (above), the team rejects species status for *M. fraseri* var. *pyramidata* since there was only one restriction site change between it and *M. fraseri* var. *fraseri* in their analysis. Also, separate allozyme profiles that were done in an earlier study (Qiu and Parks, 1994) of wild populations of *M. fraseri* at low elevations in north Georgia indicated that those plants were intermediate between var. *fraseri* and var. *pyramidata*.

For many, the major finding of this work, the sister relationship between *M. tripetala* and the Asian species (especially *M. hypoleuca*), comes as no surprise since these species share so many common morphological characters. Even the grooved seed coats (the name *Rytidospermum* means

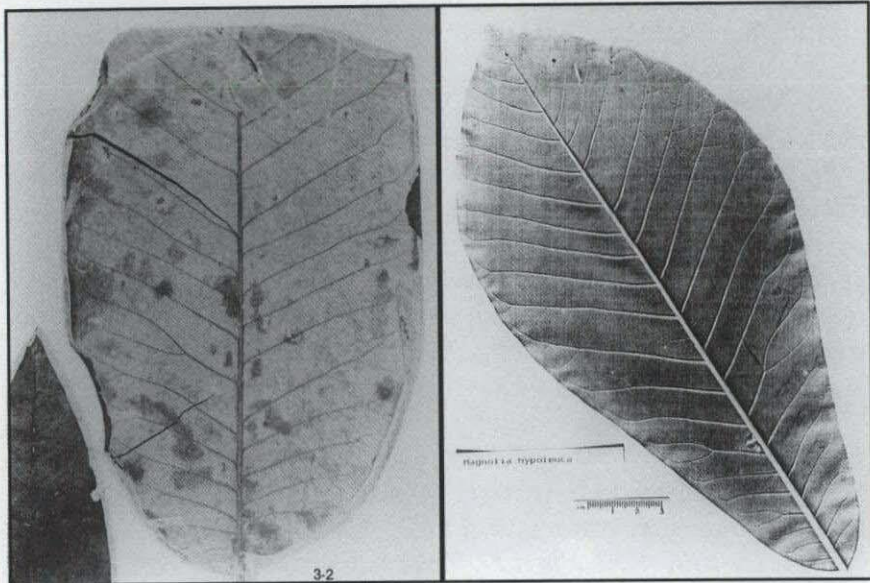


Figure 1. Rytidospermum-like fossil  
Magnolia leaf of Miocene age from  
Clarkia, Idaho.

Figure 2. Modern *M. hypoleuca* leaf.

"wrinkled seed") are shared only by *M. tripetala* and its Asian sisters. Phil Savage has indicated (pers. comm.) that of the many crosses he has made between species within section Rytidospermum, only the ones within the "sister group" were vigorous worthy hybrids. In fact, there have been many cases of putative hybrids occurring spontaneously where *M. tripetala* grows in close proximity to *M. hypoleuca* (Vasak, 1973; Spongberg and Weaver, 1981). Other crosses Savage made, which include *M. tripetala* x *M. fraseri*, *M. hypoleuca* x *M. macrophylla*, *M. tripetala* x *M. macrophylla*, and *M. hypoleuca* x *M. fraseri*, generally produced smaller leaves and flowers than their parents, and all lacked vigor. Earlier in a discussion of the interrelationships within section Rytidospermum (1976), Savage speculates that *M. hypoleuca* and *M. fraseri* could be as close as "kissin cousins" on account of their similarly beaked carpels. I must confess that I too once shared this same view. So even morphology can sometimes be tricky, and Qiu *et al.* point out that much of the morphological similarity shared by all section Rytidospermum



species could be the result of convergent evolution.

J. E. Dandy (unpublished manuscript) defines section *Rytidospermum* as consisting of three series based on morphology and biogeography—series *Umbrellae* (the North American *M. tripetala*, *M. fraseri* and its variety), series *Macrophyllae* (the North American *M. macrophylla* and its varieties), and series *Hypoleucae* (the eastern Asian *M. hypoleuca*, *M. officinalis*, and *M. rostrata*). [Note – Dandy's series definition was published in Treseder (1978) as American series 1, American series 2, and Asian series.] More recently, John D. Tobe (1993) performed a molecular systematic study of eastern North American species of *Magnolia*. His findings, like those of Qiu *et al.*, showed that *M. tripetala* did not pair with *M. fraseri* (nor with *M. macrophylla*). However, Tobe had not included the Asian *Rytidospermum* species in his study scope. Based on his findings, Tobe recommended that series *Umbrellae* be retained and to consist only of *M. tripetala*, and that the new series *Auriculatae* be created to delineate the *M. fraseri* complex.

Based on the extremely low molecular divergence shown by Qiu *et al.* between *M. tripetala* and its Asian sister taxa, along with the strong morphological and cross compatibility evidence, it is recommended that series *Hypoleucae* and *Umbrellae* be rejected and that series *Rytidospermae* be created to consist of *M. tripetala* and its Asian sister taxa. The name *Rytidospermae* is preferred since it recognizes an important common morphological trait, the unique grooved seed coats, of the species in this intercontinental group. Thus, section *Rytidospermum* can be provisionally defined by the following series:

Series *Rytidospermae*: *M. tripetala*, *M. hypoleuca*, *M. officinalis* var. *officinalis*, *M. officinalis* var. *biloba*, and *M. rostrata*.

Series *Macrophyllae*: *M. macrophylla* var. *macrophylla*, *M. macrophylla* var. *dealbata*, and *M. macrophylla* var. *ashei*.

Series *Auriculatae*: *M. fraseri* var. *fraseri*, and *M. fraseri* var. *pyramidata*.

The above seems to make sense. However, it is obvious that two of the three series are now reduced to just single species! Thus, there doesn't appear to be a need to define series here. What is really needed is a complete taxonomic re-evaluation of section *Rytidospermum*. ☞

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