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Table of Contents:

THE WORLD OF PEONIES ACCORDING TO SANG

by Don Smith

Creation of new species (Speciation) through natural hybridization, especially when combined with polyploidization, is an important mechanism in plants and it has made the science of classifying plants (Taxonomy) a very challenging field of study. This mechanism is known as reticulate evolution. Until quite recently, nearly all plant classifications were made solely on the basis of morphological characteristics and differences. As a result of this, taxonomy has remained a relatively inexact science, with considerable room for differences of opinion and frequent debates among taxonomical experts. Classification has been particularly difficult in peonies and other plant groups, which have undergone extensive reticulate evolution. These difficulties have resulted in numerous adjustments, changes and reclassifications over the years. Recent advances in modern genetics however, have made taxonomy a much more precise science, with significantly less room for error and ambiguities. For example, the recent application of molecular markers has greatly facilitated the detection of hybridization and the recognition of allopolyploids in many plant groups. This is especially true in Pæonia, where a recent landmark study by Tao Sang (1995) has clearly established that reticulate evolution (hybridization) has played a primary role in speciation in peonies. In his doctoral dissertation at Ohio State University entitled “Phylogeny and

Biogeography of Pæonia (Pæoniaceae)", Dr. Sang has laid the foundation for a completely new and much more accurate reclassification of section Pæonia (i.e., Pæon) of Genus Pæonia.

In the latest and most widely recognized monograph of Pæonia, Stern (1946) divided the Genus into three sections, Moutan, Onaepia, and Pæon (Pæonia). He further divided section Pæon into two sub-sections, Foliolatae and Dissectifoliae, based on the degree of dissection of the foliage. Stern placed 20 species into sub-section Foliolatae and the remaining 8 species in sub-section Dissectifoliae. The classification of section Pæonia according to Stern is shown in Table 1. For comparison, I have also included two other "classifications" that have appeared in recent books on Peonies. At the bottom of the table, I have also added three newly discovered species that were not classified by Stern.

Table 1. Summary of Previous Classifications of Genus Pæonia, Section Pæonia (Pæon)

Species (Stern, 1946)	New Species, New Name or Current Status	Sub-Section Assignment (Stern, 1946)	Sub-Section Assignment (Cooper, 1988)¶	Sub-Section Assignment (Page, 1997)*
Mlokosewitschi		Foliolatae	Foliolatae	Foliolatae
Wittmanniana		"	"	"
Cambessedesii		"	"	"
Russi	Masc. ssp. russi	"	"	"
Mascula	Masc. ssp. masc.	"	"	"
Corallina	Syn. with Mascula	"	"	"
Daurica	? ssp. of Mascula	"	"	"
Banatica	ssp. of Officinalis	"	"	Dissectifoliae
Kesrouanensis	? ssp. of Mascula	"	"	Dissectifoliae
Obovata		"	"	Foliolatae
Japonica		"	"	Dissectifoliae
Mairei		"	"	Foliolatae
Oxypetala	ssp. of Mairei	"	"	-
Arietina	Masc. ssp. arietina.	"	"	Foliolatae
Rhodia		"	"	Dissectifoliae
Bakeri	? form of Arietina	"	"	Foliolatae
Broteri		"	"	Foliolatae
Coriacea		"	"	Dissectifoliae
Lactiflora		"	"	Foliolatae
Emodi		"	"	Foliolatae
Peregrina		Dissectifoliae	Dissectifoliae	Dissectifoliae
Officinalis		"	Foliolatae	"
Clusii		"	Foliolatae	"
Humilis	ssp. of Officinalis	"	Foliolatae	"
Mollis	Sterile(prob. hyb.)	"	Foliolatae	"
Tenuifolia		"	Dissectifoliae	"
Anomala		"	Dissectifoliae	"
Veitchii		"	Dissectifoliae	"
	Xinjiangensis		Dissectifoliae	-
	Sterniana		Dissectifoliae	Dissectifoliae
	Parnassica		Foliolatae	"

* from **The Gardener's Guide to Growing Peonies** by Martin Page, Timber Press (1997);
¶ from **Peonies** by Allan Rogers, Timber Press (1995)

In the next few pages I will try to summarize the methods, results and conclusions of the Sang study. The details of the methods used are extremely technical and are way over my head. The results and conclusions, however, can be easily described in laymen's terms and therefore I will concentrate on these aspects of the study.

The analysis of DNA sequences can be an exrtremely powerful tool in reconstructing reticulate evolution. Sang analyzed two different types of DNA sequence data to help reconstruct the very complex pattern of reticulate evolution in section Pæonia. First he analyzed nucleotide additivity detected by directly sequencing PCR products of internal transcribed spacers (ITS) of nuclear ribosomal DNA. This method can be highly informative and accurate in detecting hybridization in cases where parental sequences are maintained in the hybrid species, which is apparently the case in peonies due to the slowing of concerted evolution caused by the long generation time of peonies. Next, Sang analyzed the rapidly evolving (maternally inherited) chloroplast gene, matK. This technique can identify the maternal parent of a hybrid species (and thus when combined with ITS sequence data, also the paternal parent by the process of elimination).

By analyzing both nuclear and chloroplast DNA sequences, Sang was able to accurately reconstruct the complex reticulate evolution patterns within section Pæonia. However, he also emphasizes that this reconstruction may still be an underestimate of the natural hybridization which has occurred in this group. Probably the most surprising conclusion of the study was the high proportion of diploids among the hybrid species. Although the tetraploid species have often been suggested to be allotetraploids (thus indicating hybrid origin), the diploid species of this section have never previously been considered to be of hybrid origin.

In summary, this technique can:

1. Detect hybridization events (including relatively ancient events and multiple hybridization events in the same lineage).
2. Identify the parent species of each hybrid species.
3. Identify the maternal and paternal parents (including extinct species and ancient ancestors) of many hybrid species.
4. Identify closely related species that share a common parentage thus forming a sister group.
5. Determine the relative order in which various hybridization events occurred.
6. Determine the approx. time that a certain hybridization event took place (i. e., determine the time of origin of the new species).

In the remainder of this article I will attempt to summarize the more important conclusions of the Sang study.

The key conclusions are:

? Extensive reticulate evolution in section Pæonia has made natural classification very difficult.

? Phylogenetic analysis supports recognition of three taxonomic sub-sections in section Pæonia (rather than only two).

? The majority of the species in section Pæonia are of hybrid origin.

? Most hybrid species are found in the Mediterranean region, whereas their parental species are presently restricted to Asia.

? The earliest evolutionary split in genus Pæonia probably occurred between section Onaepia and the rest of the genus, thus making Onaepia the oldest section. It is estimated that this split occurred 13.3 million years ago.

? P. anomala, P. lactiflora and P. veitchi are probably the only species in section Pæonia not derived from hybridization.

? The proportion of diploids among the hybrid species is surprisingly high, suggesting that hybrid speciation at the diploid level has been quite successful in peonies.

The results of Sang's phylogenetic analysis of section Pæonia are summarized in Figure 1, (which is Sang's figure 6). Using this figure, we can trace the reticulate evolution within section Pæonia by following the evolutionary tree from left to right.

In ancient times, following the initial split of section Onaepia from the rest of the genus and the subsequent split of sections Moutan and Pæonia (which are not shown in the figure), section Pæonia evolved into two distinct clades, a large clade (top of figure) and a small clade (bottom). The original ancestors of these two clades hybridized to create a new hybrid species, which in turn, replaced its paternal parent to become the only surviving ancestor of the small clade. After this, extensive hybridization occurred between members of the two clades and also between members within the large clade. Many of the resulting hybrids survived and evolved into new species, which in a few cases replaced one of their parental species. Some accomplished this through polyploidization, while others managed to survive by remaining at the diploid level. This probably occurred through extensive vegetative reproduction by rhizomes which facilitated survival of the initial diploid populations of hybrids until they became fertile or polyploidized.

Based on figure 1, the species of section Pæonia can be grouped into hybrid and non-hybrid species as follows:

Non Hybrid Species

Anomala
Veitchi
Lactiflora

Hybrid Species

The hybrid species can be divided into two types, Interclade (Intersub-sectional) hybrids and Intraclade (Intrasub-sectional) hybrids. The interclade hybrids are hybrids between parental species representing each of the two clades (sub-sections), whereas the intraclade hybrids are the progeny of hybridization events where both parents are from the large clade. The species in each category as determined from figure 1 are given below.

Intersub-sectional Hybrid Species

Russi
Cambessedesii
Banatica
Clussi
Rhodi
Broteria
Coriacea
Mloko
Mascula
Sterniana
Obovata
Japonica
Mairei

Intrasub-sectional Hybrid Species

Emodi
Xinjiangensis
Peregrina
Tenufolia
Wittmanniana

Probable Hybrid Species of Unknown Origin*

Arietina
Humilis
Officinalis
Parnassica

* Since these species have been determined to be allotetraploids based on meiotic evidence (Stebbins, 1948) and cytogenetic studies (Tzanoudakis, 1977; Schwarzacher-Robinson, 1986), they are considered to be of hybrid origin, even though the phylogenetic evidence to support this conclusion is still lacking. They could be either interclade or intraclade hybrids and one or both of the parental species may be extinct.

Figure 1 clearly indicates that the majority of the species in section Pæonia are the result of one or more hybridization events. Based on his analysis, Sang recommends classification into three sub-sections, with one section containing the non-hybrid Asian species with dissected leaves and the hybrid species derived from them (i. e., P. anomala, P. veitchi, P. lactiflora, P. emodi, P.

xinjiangensis). The second sub-section would contain the eastern Asian species with fewer and broader leaflets (i. e., P. mairei, P. obovata, P. japonica). The third sub-section would contain all the Mediteranean species that have been derived through hybridization between the first two sub-sections. There is no specific mention of the placement of the sister group containing the four tetraploid species, arietina, humlis, officinalis and parnassica. It should also be noted that, although mairei, obovata and japonica are the purest existing representatives of the small clade, they too are also hybrid species, which have been derived through hybridization between relatively ancient members of the two clades (see fig. 1). In each case the paternal parents of these hybrid species appear to be extinct.

Classification of Section Pæonia based on the recent study by Sang (1995)

Sub-section Pæonia

Anomala
Veitchi
Lactiflora
Emodi
Xinjiangensis

Sub-section Foliolatae

Mairei
Obovata
Japonica
Banatica

Sub-section Intermedia

Russi
Cambessedesii
Clussi
Rhodi
Broteria
Coriacea
Mloko
Mascula
Sterniana
Wittmanniana
Peregrina*
Tenufolia*
Arietina*
Humilis*
Officinalis*
Parnassica*

*Since the origin of the last four species is unknown and could not be determined by the phylogenetic analysis of Sang, the placement of these four species remains uncertain. If they originated by hybridization within the large clade only (i.e., they are intraclade hybrids), then they belong in sub-section Pæonia with the other intraclade hybrids such as Emodi and Xinjiangensis. On the other hand, if they evolved instead through hybridization with a species in the small clade, then they belong in the intermedia sub-section with the other interclade hybrids, where I have placed them. It is entirely possible that the parents of these four "sister" species are ancient species which have long been extinct. As a result of this uncertainty, the placement of Tenufolia and Peregrina, two species derived from the "four sisters" group, is also uncertain.

Other conclusions that can be drawn from figure 1 are as follows:

? The small clade has no pure species (non-hybrid species), since the entire clade evolved from a very ancient hybrid between an ancestor of the large clade and a long extinct ancestor of the small clade.

? The maternal parent of tenufolia is most likely an extinct species in the large clade. The paternal parent of the sister species, obovata and japonica is probably an extinct species in the small clade.

? Emodi and Xinjiungensis are hybrid species with the same parents, but in the reverse order.

Xinjiungensis = Lactiflora x Veitchi

Emodi = Veitchi x Lactiflora

? Of the extant species in section Pæonia, Anomala and Mairei are the most ancient, followed by Lactiflora and Veitchi. Whereas Clussi, Rhodi, Broteri, Corciacea, Mascula and Mloko. are the most ancient of the species of hybrid origin, followed by Obovata, Japonica and Xinjiungensis. On the other hand, Sterniana is the most recent species, followed by Banatica and then Emodi and Wittmanniana.

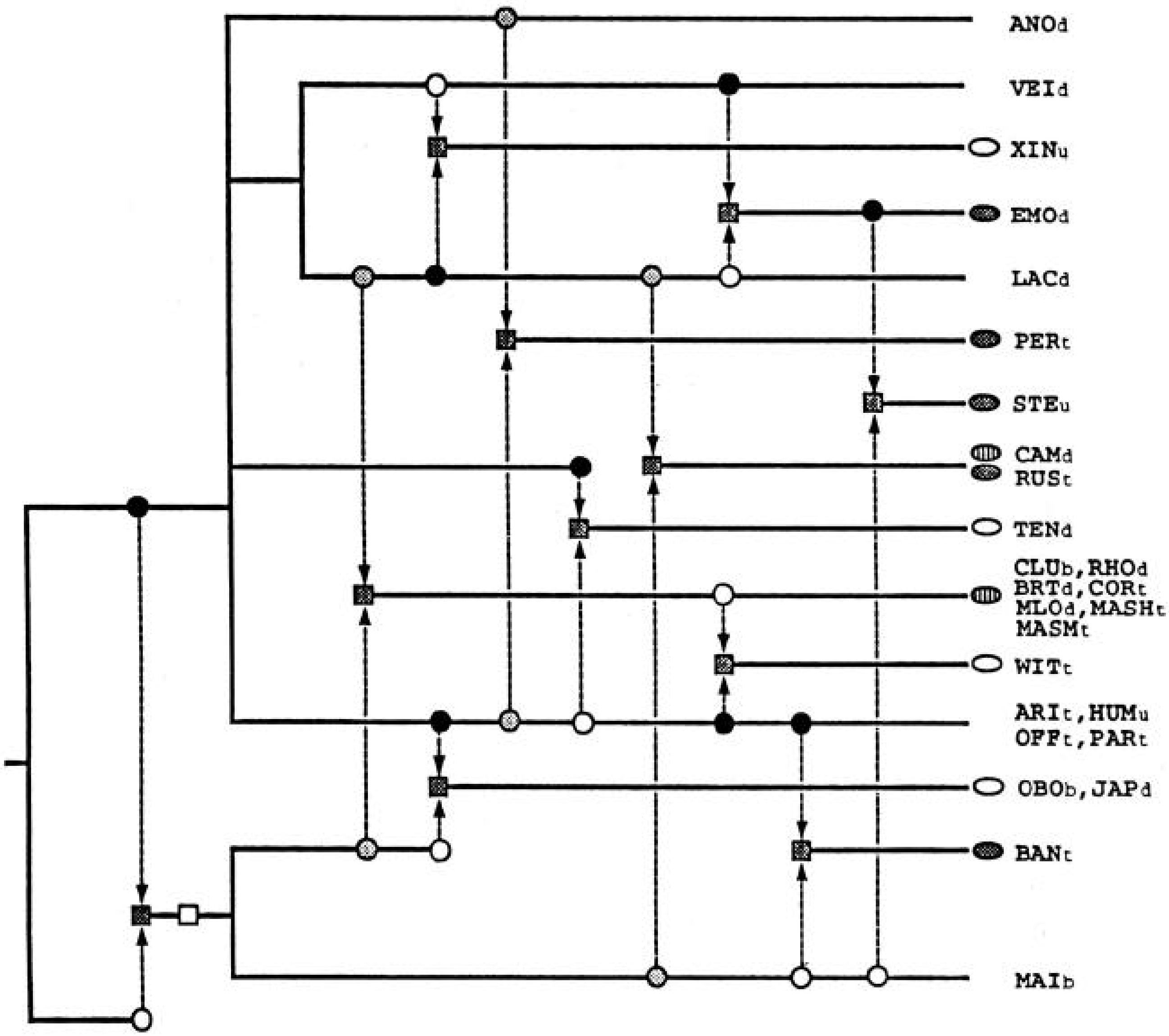

Figure 1. This figure summarizes the results of Sang's analysis of section Pæonia. It appears in his thesis as Figure 6. The following is the caption as it appeared with this figure. Fig. 6. Phylogeny of Paeonia section Paeonia reconstructed from a synthesis of the ITS and matK phylogenies. Solid lines represent divergent and patristic evolution, but length of lines is not proportional to amount of patristic change. Dashed lines represent reticulate evolution. Solid circles, maternal parents; open circles, paternal parents; shaded circles, parents with uncertain maternity or paternity; shaded squares, hybrids; open square, a hybrid with fixed ITS sequences similar to its paternal parent; open ellipse, hybrid species with fixed paternal ITS sequences; shaded ellipse, hybrid species with fully additive ITS sequences from their parents; striped ellipse, hybrid species with partially additive ITS sequences from their parents. An attempt is made to indicate the relative order of occurrence of hybridizations, but it may not be totally accurate. Hybrid species completely fixed for one parental type ITS sequence or with partially additive ITS sequences are considered to have a more ancient origin than species with full ITS additivity if we assume that gene conversion operated at a relatively constant rate over all hybrids. Hybrid species identified by ITS additivity but without maternal parents identified by matK sequences are considered to have a more ancient origin than those sharing matK mutations with their maternal parents if we assume that lack of shared matK mutations is due to occurrence of hybridization prior to accumulation of mutations in the maternal parents. Species abbreviations as in Fig. 1. d = diploid; t = tetraploid; b = both diploid and tetraploid populations known; u = ploidy level unknown.